Novel therapeutic concepts

Genetics of inherited cardiomyopathy

Daniel Jacoby¹ and William J. McKenna²*

¹Division of Cardiology, Yale School of Medicine, New Haven, CT 06519, USA; and ²Institute of Cardiovascular Science, University College London and The Heart Hospital, UCLH NHS Trust, 16-18 Westmoreland Street, London W1G 8PH, UK

Received 20 January 2011; revised 20 June 2011; accepted 6 July 2011; online publish-ahead-of-print 2 August 2011

During the past two decades, numerous disease-causing genes for different cardiomyopathies have been identified. These discoveries have led to better understanding of disease pathogenesis and initial steps in the application of mutation analysis in the evaluation of affected individuals and their family members. As knowledge of the genetic abnormalities, and insight into cellular and organ biology has grown, so has appreciation of the level of complexity of interaction between genotype and phenotype across disease states. What were initially thought to be one-to-one gene-disease correlates have turned out to display important relational plasticity dependent in large part on the genetic and environmental backgrounds into which the genes of interest express. The current state of knowledge with regard to genetics of cardiomyopathy represents a starting point to address the biology of disease, but is not yet developed sufficiently to supplant clinically based classification systems or, in most cases, to guide therapy to any significant extent. Future work will of necessity be directed towards elucidation of the biological mechanisms of both rare and common gene variants and environmental determinants of plasticity in the genotype-phenotype relationship with the ultimate goal of furthering our ability to identify, diagnose, risk stratify, and treat this group of disorders which cause heart failure and sudden death in the young.

Keywords
Cardiomyopathy • Genetics

Introduction

Cardiomyopathies are a clinically heterogeneous group of heart muscle disorders. They are defined by the presence of abnormal myocardial structure and/or function in the absence of ischaemic heart disease or abnormal loading conditions. The current classifications of the cardiomyopathies continue to be based on phenotype defined by clinical evaluation of affected individuals, incorporating genotype when possible.

While differences exist in the classification schema of major cardiac organizations (e.g. pure channelopathies are included in the American classification system), the cardiomyopathies have historically broken down into several major phenotypic categories: hypertrophic, dilated, arrhythmogenic, and restrictive. (Figure 1).¹²

Overlap of the phenotypes is common, i.e. the patient with hypertrophic cardiomyopathy (HCM) may have restrictive physiology. With disease evolution, there also may be progression from one phenotype to another. Since the identification of the first cardiac disease-causing mutation, a point mutation in the β-myosin heavy chain gene in a French Canadian family with HCM in 1990,³⁴ in excess of 600 rare genetic variants associated with cardiomyopathic disease have been recognized.⁵⁶ Variable penetrance with incomplete expression is common in the autosomal dominant forms of cardiomyopathy, even among related individuals carrying an identical gene mutation. This combination of phenotypic and mutational heterogeneity contributes importantly to the challenges in diagnosis and prognostication, and the complexity of treatment. Additionally, genetic diagnosis represents a moving target with new data leading to reevaluation of mutation pathogenicity. Finally, modifier gene and environmental effects are increasingly appreciated as key components of this genotype-phenotype plasticity.

The current body of knowledge on genetics of cardiomyopathy suggests a basis for understanding the pathophysiology of disease, provides potential targets for therapeutic intervention, contributes to diagnosis, allows for cascade screening, and occasionally informs prognosis. Used appropriately, genetic testing can provide important additional information for patients and their families.

This review will focus on the genetic basis of the cardiomyopathies, models of pathogenesis associated with known mutations, and the utility and yield of genetic testing. Specifically, we aim to highlight the current state-of-the-art understanding of the genetics of cardiomyopathy as it impacts the clinical presentation of these
diseases. It is necessarily limited in scope but will highlight the key strengths and limitations of our current understanding of the genetics of cardiomyopathy and the determinants of genotype–phenotype plasticity (Table 1).

Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy is characterized by myocardial hypertrophy in the absence of clinically important abnormal loading conditions or primary valve disease. Diagnosis relies on electrocardiographic (ECG) evidence of left ventricular hypertrophy (LVH) confirmed by two-dimensional echocardiography or magnetic resonance imaging. Histological abnormalities are typified by myocyte disarray, interstitial and replacement fibrosis, and medial hypertrophy of intramyocardial small vessels. Clinical concerns in patients with HCM include development of dynamic obstruction of the left ventricular outflow tract (LVOT), atrial and ventricular arrhythmias complicated by either stroke or sudden cardiac death (SCD), and progression to diastolic and/or systolic heart failure (HF). Familial forms of HCM typically exhibit an autosomal dominant inheritance pattern, with sporadic disease and phenocopies accounting for a majority of cases of non-sarcomeric disease.

Familial HCM is characterized by marked phenotypic heterogeneity, age-dependent penetrance, and variable expression. Negligible to extreme hypertrophy, with varying degrees of fibrosis and outflow tract obstruction, can be seen within a single family. The population prevalence of clinically identifiable HCM is estimated to be 1:500 but this probably underestimates the prevalence of genetic disease. While the clinical course is relatively benign for a majority of those with HCM, the risk of sudden death is ≏1% per year for adults. A significant minority (2–5%) may develop progressive HF from a combination of pump failure and/or restrictive physiology, which can be further complicated by atrial arrhythmias and stroke.

Left ventricular outflow tract obstruction is present at rest in ≏35%, may develop during exercise in another subset, and provides an important target for treatment in some symptomatic patients. The mechanisms of obstruction relate to septal thickness, LVOT dimensions, and mitral valve/papillary muscle anatomy. None of these features has a direct genetic correlate. Covariant and modifier genes not yet identified may play a role.

Sudden cardiac death is a primary clinical concern in the care of patients with HCM. While the absolute risk of SCD in all comers is low, it remains an important cause of SCD in the young and otherwise healthy. Risk factors predictive of SCD risk are relatively well established with increasing levels of risk determined by the number and severity of factors (Figure 2). Familial evaluation in HCM may serve to identify affected individuals who, though asymptomatic, are at risk for SCD. Risk assessment and consideration of lifestyle modification with or without an implantable cardiac defibrillator (ICD) underpins the rationale for family
screening and ongoing clinical monitoring of affected individuals. Recommendations for lifestyle modification, such as avoidance of competitive sports, depend largely on phenotype but in specific cases (see below) may be impacted by genotype even in phenotype-negative individuals. Because of the complexity of the genotype and phenotype relationship, accurate prognostication based on genetic data is currently elusive, but remains a future goal.

### Genetics of hypertrophic cardiomyopathy

Since the original genetic discovery, in excess of 400 mutations in nine genes encoding functionally important proteins of the cardiac sarcomere have been identified in association with HCM accounting for the majority of mutations found in clinical cohorts with this disease. The distribution of pathogenic sarcomeric mutations is uneven with myosin heavy chain 7 (MYH7) and myosin-binding protein C3 (MYPBC3) making up ≈25% each, and cardiac troponin T (TNNT2), cardiac troponin I (TNNI), myosin ventricular regulatory light chain (MYL2), and myosin ventricular essential light chain (MYL3) accounting for most of the remainder. Attempts to look beyond the sarcomeric proteins for genetic causes of HCM are driven by the recognition that 30–40% of the patients with clinical HCM do not have sarcomere mutations. Interest in a growing number of genes potentially associated with HCM broadly characterized as Z-disc/sarcomere genes and genes of calcium handling has emerged. The prevalence and pathogenicity of these mutations in studied populations awaits confirmation. It is possible, even likely, that some of these genes serve mainly as modifiers of primary mutations.

Five to 10% of the patients identified in cohort screenings carry multiple sarcomeric mutations and a dose–effect relationship occurs, with compound heterozygotes tending to present with more severe disease at an earlier age. As a result, more extensive genetic evaluation may be warranted in probands presenting with early or severe disease, especially in cases where the severity of disease significantly exceeds that seen in other family members.

### Table 1 Genetic terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband:</td>
<td>The first individual in a family who presents with clinical disease, sometimes referred to as the index case</td>
</tr>
<tr>
<td>Phenotype:</td>
<td>The observable characteristics of an individual</td>
</tr>
<tr>
<td>Genotype:</td>
<td>The genetic make-up of an individual</td>
</tr>
<tr>
<td>Mutation:</td>
<td>A pathogenic gene variant. Mutations have a tendency to be rare, to occur in conserved or functionally important regions of the gene, to segregate with observable disease, and to occur where there is biological plausibility that the involved gene could lead to the observed phenotype</td>
</tr>
<tr>
<td>Modifier:</td>
<td>Gene variants or environmental factors that are insufficient to cause observable disease on their own, but which are capable of interacting with the disease gene to alter the phenotype</td>
</tr>
<tr>
<td>Heterozygote:</td>
<td>An individual who carries a single copy of a mutation</td>
</tr>
<tr>
<td>Homozygote:</td>
<td>An individual who carries two copies of a mutation</td>
</tr>
<tr>
<td>Age-dependent expression:</td>
<td>The tendency to develop more observable or severe phenotypes with advancing age</td>
</tr>
<tr>
<td>Variable penetrance:</td>
<td>Variability in the proportion of genotypically identical individuals who express the disease phenotype</td>
</tr>
<tr>
<td>Variable expression:</td>
<td>Variability in observable characteristics among carriers of an identical mutation</td>
</tr>
<tr>
<td>Phenotypic heterogeneity:</td>
<td>Phenotypic variability among individuals with similar genotypes</td>
</tr>
<tr>
<td>Genotypic heterogeneity:</td>
<td>Genetic variability among individuals with similar phenotypes</td>
</tr>
<tr>
<td>Genotype–phenotype plasticity:</td>
<td>The concept that the link between genotype and phenotype is subject to broad variability with as yet limited predictability</td>
</tr>
<tr>
<td>Phenocopy:</td>
<td>A phenotype that mimics the disease phenotype, but having a different aetiology (genetic or environmental), clinical course, and/or systemic features</td>
</tr>
</tbody>
</table>

---

**Figure 2** Risk factors for sudden cardiac death in hypertrophic cardiomyopathy.
This speaks particularly strongly to the importance of family assessment, as most patients with multiple mutations will have inherited disease from both maternal and paternal lineages.

Although effort has gone into relating genotype and phenotype with the aim of supplementing clinically based diagnostic and risk factor assessment algorithms, at present, the data sets on specific mutations are not significant enough to be a major clinical guide. Specific mutations in MYH7 (Arg430Gln, Arg453Cys, and Arg719Trp) appear convincingly associated with adverse outcomes; however, data suggests that at-risk patients carrying these mutations also display clinical risk factors at the time of events limiting the added prognostic benefit of genetic diagnosis. An exception to this is HCM caused by mutations in cardiac troponin T which may cause ventricular arrhythmias and SCD in the absence of impressive morphological (LVH) or haemodynamic features (obstruction, diastolic dysfunction). In this context, knowledge that disease is caused by a TNNT mutation may influence management with a lower threshold for prophylactic ICD implantation.

The pathogenesis of HCM associated mutations is incompletely worked out but important pathogenic mechanisms have been elucidated. During normal contraction, calcium binding to α-tropomyosin and the troponin complex leads to removal of inhibitory troponin I. This allows association of the myosin head (a component of the thick filaments) with actin (a component of the thin filaments) and ATP hydrolysis with consequent conformation change in the myosin neck and sliding of thin filaments in relation to thick filaments. Basic science investigations incorporating mutated MYH7 demonstrate increased force generation and more rapid actin–myosin cross-bridge sliding. Similarly, mouse models of α-cardiac myosin and troponin T mutations demonstrate altered energy dynamics with increased force generation and abnormal relaxation properties. Additionally, reduced energy efficiency in sarcomere force development has been demonstrated in transgenic rat models, while in humans, markers of altered cellular energy dynamics (phosphocreatine to ATP ratio) have been noted in ~30% of HCM patients independent of the degree or presence of hypertrophy. The pathways linking altered energy dynamics at the sarcomeric level with development of gross hypertrophy remain incompletely elucidated but alterations in intracellular calcium handling and regulation of calcium-sensitive signalling proteins appear to play a role. Based on these and other observations, clinical investigation into the use of diltiazem for prevention of hypertrophy in genotype-positive, phenotype-negative individuals is ongoing.

Phenocopies of HCM are seen in a sizeable minority of patients presenting with idiopathic hypertrophy. Protein deposition disease (amyloidosis) and glycogen storage diseases (systemic and isolated cardiac) are most common but vary in prevalence with the population being tested. Fabry’s disease, Danon’s disease, Pompe’s disease, and Noonan’s syndrome can all present with ventricular hypertrophy that may be difficult to distinguish from sarcomere-associated HCM on a clinical basis alone, though histopathological findings are usually distinct. Hypertrophy seen in the setting of true pre-excitation is associated with SCD. This is important to note that the hypertrophy seen in these metabolic diseases represents true hypertrophy of the cardiomyocyte where the proportion of increased cardiac mass attributable to glycogen deposition is minimal. Thus, ECGs of these patients typically demonstrate dramatically increased QRS voltage and major repolarization changes.

Utility of genetic testing in hypertrophic cardiomyopathy

A genetic diagnosis is obtainable in 60–70% of consecutive patients with familial HCM, though the yield is lower (~30%) when sporadic disease is considered. As mentioned, troponin T mutations have been associated with SCD in the absence of traditional risk factors and identification of such a mutation should lead to consideration for early ICD placement. For other mutations, even those associated with severe disease, SCD rarely occurs in the absence of clinical risk factors making the added utility of genetic diagnosis in this group minimal. Those with special characteristics such as conduction disease, pre-excitation, or systemic disease may represent phenocopies of sarcomere-associated HCM and focused genetic testing may be helpful with diagnosis and treatment (e.g. Danon’s disease, PRKAG2).

Although studies evaluating prognostic significance of mutational analysis in HCM have failed to demonstrate consistent outcomes, a family history of SCD remains an important risk factor, indicating that genetic background may yet prove helpful in risk assessment. Importantly, a large cohort study of unrelated patients with HCM demonstrated that a positive genetic diagnosis for any myofilament mutation was associated with quadruple the risk of adverse outcomes including cardiovascular death, stroke, and progression to advanced HF, in comparison with those who were found to be genotype-negative. However, because no specific action or change in therapy can be recommended for any individual patient based on this finding, the relevance of this observation to clinical gene testing is limited.

The potential for medical therapy to attenuate or prevent disease development in pre-clinical genotype-positive individuals has yet to be realized though early investigations in humans and animals demonstrate some promise. The greatest benefit of genetic testing in HCM derives from cascade screening with the ability to identify which individuals in a family are, or are not, at risk of disease development. Cascade screening is possible/feasible when a pathogenic mutation is identified in a proband and there are ‘at risk’ family members. Those carrying the mutation can be followed closely for the development of disease and counselled appropriately with regard to lifestyle, while those who do not carry the mutation may be released from follow-up (a cost-effective approach). For cascade screening to be safe and effective, it is critical that the pathogenicity of the mutation be certain. Because many mutations that cause HCM are private (unique to the individual family), careful family assessment demonstrating co-segregation of mutation with disease phenotype is advised.

Arrhythmogenic cardiomyopathy/arrhythmogenic right ventricular cardiomyopathy

The clinical spectrum of arrhythmogenic cardiomyopathy (ACM) is variable but is typified by electro-anatomical abnormalities,
Disease phenotypes include the classic right-dominant form known as arrhythmogenic right ventricular cardiomyopathy (ARVC), as well as increasingly noted left-dominant and biventricular forms. While dysfunction of the right ventricles and/or LVs is common in later stages, the disease typically presents with ECG abnormalities and ventricular arrhythmias. Desmosomal gene mutations have been identified in association with all three subtypes leading to an appreciation for the broad spectrum of desmosomal gene expression. While familiar, the term ARVC no longer accurately reflects the breadth of phenotypes reported within this cardiomyopathy. Accordingly, the term ACM has been accepted by the HRS/EHRA Expert Consensus Statement on the State of Genetic Testing for Channelopathies and Cardiomyopathies (in press).

The clinical profile of ACM is outlined in Table 2. Updated diagnostic guidelines incorporating anatomic, histological, electrophysiological, arrhythmic, and genetic features have been proposed for arrhythmogenic ventricular cardiomyopathy (AVC). The updated classification system addresses the recognition that early or familial disease may be overlooked prior to previous criteria that were derived largely from index cases presenting with sustained ventricular arrhythmia or advanced structural disease (right ventricular failure and/or SCD). Importantly, early disease, manifested by subtle structural abnormalities and minor ventricular arrhythmias, may be associated with SCD, highlighting the importance of disease identification during this so-called ‘concealed’ phase.

The prevalence of ACM is thought to be ~1:1000 though, as with other cardiomyopathies, age-dependent penetrance and variable expression makes the true prevalence difficult to ascertain. A minority of patients progress to clinically important ventricular dysfunction with the clinical hallmark of disease remaining ventricular arrhythmias. Inheritance patterns are mainly autosomal dominant but well-described rare recessive forms are observed and have been of significant import in the development of genetic and pathophysiological understanding in this disease.

Table 2 Clinical profile of arrhythmogenic ventricular cardiomyopathy\(^a\) (reproduced from Sen-Chowdhry et al.\(^{58}\))

<table>
<thead>
<tr>
<th>Diagnostic measure</th>
<th>Classic right dominant</th>
<th>Left dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-Lead ECG</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Poor R-wave progression(^b)</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>IVCD in V1–3</td>
<td>Leftward QRS axis ((30^\circ \leq \text{QRS axis} &lt; 0)) or left-axis deviation ((\text{QRS axis} &lt; -30^\circ))</td>
</tr>
<tr>
<td></td>
<td>Prolongation of QRS duration in V1–3</td>
<td>Early transition</td>
</tr>
<tr>
<td></td>
<td>Incomplete RBBB</td>
<td>LBBB</td>
</tr>
<tr>
<td></td>
<td>RBBB</td>
<td>Epsilon waves in inferior (II, III, aVF) and/or lateral leads (V5–V6 ± V4, I, aVL)</td>
</tr>
<tr>
<td></td>
<td>Epsilon waves in V1–3</td>
<td>Inverted/flat T-waves in (infero)lateral leads, extending to V1–3 with LV involvement</td>
</tr>
<tr>
<td></td>
<td>Inverted/flat T-waves in V1–3, extending to V4–6 with LV involvement</td>
<td>Inverted/flat T-waves in (infero)lateral leads, extending to V1–3 with RV involvement</td>
</tr>
<tr>
<td></td>
<td>ST elevation in V1–3</td>
<td>ST elevation in V1–3</td>
</tr>
<tr>
<td>Signal-averaged ECG</td>
<td>Late potentials</td>
<td>Late potentials</td>
</tr>
<tr>
<td>Arrhythmia</td>
<td>Both supraventricular tachycardia and atrial fibrillation/flutter are observed in arrhythmogenic cardiomyopathy but are not contributory to diagnosis</td>
<td>Both supraventricular tachycardia and atrial fibrillation/flutter are observed in arrhythmogenic cardiomyopathy but are not contributory to diagnosis</td>
</tr>
<tr>
<td>Ventricular volumes</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Sustained/non-sustained VT of LBBB configuration(^c)</td>
<td>Sustained/non-sustained VT of RBBB configuration(^c)</td>
</tr>
<tr>
<td></td>
<td>Mild, moderate, or severe RV dilation ± dysfunction</td>
<td>Mild, moderate, or severe LV dilation ± dysfunction</td>
</tr>
<tr>
<td>RV/LV volume ratio</td>
<td>≥1.2, increases with disease progression</td>
<td>&lt;1, diminishes with disease progression</td>
</tr>
<tr>
<td>Other imaging abnormalities</td>
<td>Localized dilation, WMA, and/or aneurysms in RV, preferentially affecting triangle of dysplasia and mid-free wall</td>
<td>Localized dilation, WMA, and/or aneurysms in LV</td>
</tr>
<tr>
<td></td>
<td>Increased/abnormal trabeculation</td>
<td>Increased/abnormal trabeculation</td>
</tr>
<tr>
<td></td>
<td>Fat/late enhancement in RV myocardium</td>
<td>Non-compacted appearance</td>
</tr>
</tbody>
</table>

\(\text{IVCD, intraventricular conduction delay; LBBB, left bundle branch block; PVC, premature ventricular complex; RBBB, right bundle branch block; VT, ventricular tachycardia; WMA, wall motion abnormality.}\)

\(\text{\(^a\)The clinical picture in the biventricular subtype is generally a composite of right- and left-dominant features, with RV to LV volume ratio remaining \(\approx 1\) throughout the disease course.}\)

\(\text{\(^b\)Poor R-wave progression is the primary ECG abnormality observed in the Newfoundland founder population, in which LV structural abnormalities are prominent, but the subtype of arrhythmogenic cardiomyopathy is still being elucidated. It has also been reported in \(\sim 10\%\) of patients in a cohort including all three subtypes of arrhythmogenic cardiomyopathy.}\)

\(\text{\(^c\)Non-sustained VT is defined as three or more consecutive beats at a rate of \(>120\) b.p.m.; sustained VT has a duration of \(>30\) s.}\)
Initial problems with the determination of the genetic basis of ACM were overcome by the recognition of recessive families with severe right-dominant disease and associated cutaneous manifestations (e.g. kinky hair, palmoplantar hyperkeratosis). Identification of a 2 bp deletion in JUP-encoded plakoglobin in Naxos disease was followed by identification of a point mutation in DSP-encoded desmoplakin in the Carvajal syndrome. These discoveries provided the basis to speculate that AVC is a desmosomal disease. Using a candidate gene approach, rare variants in five genes involving structural components of the cardiac desmosome have been identified in association with autosomal dominant AVC: PKP2-encoded plakophilin-2, DSG2-encoded desmoglein, DSC2-encoded desmocollin, JUP-encoded plakoglobin, and DSP-encoded desmoplakin.

Arrhythmic and structural abnormalities observed in AVC may be explained in part by disruption of force transmission, intercellular communication, and cell proliferation/differentiation. The mechanism behind these effects is increasingly appreciated to be dependent not only on primary abnormalities of the desmosome but also on the close association between desmosomes, gap junctions, and adherens junctions. Physiologically, important crosstalk exists between the cardiac desmosome, and gap and adherens junctions, with the resulting integrity of the intercalated disc and its important role in both mechanical and electrical cellular stability dependent on adequate functioning of all three subunits.

Components of cardiac desmosomes also play an important role in direct cellular signaling and proliferation/differentiation. In the presence of cardiac-specific suppression of desmoplakin, plakoglobin undergoes nuclear translocation with consequent transdifferentiation of cardiomyocytes to adipocytes. Identification of fatty replacement on histopathological examination of cardiac tissue from affected patients may be explained by this pathway, or alternatively by cellular damage and necrosis accompanied by inflammation resulting in collagen deposition, fibrosis, and/or adipose formation. Finally, desmosomes play an important role in anchoring and function of ion channels potentially impacting cellular gradients and arrhythmogenesis.

Non-desmosomal genes implicated in the development of AVC include transforming growth factor B3 (TGF3) and transmembrane protein 43 (TMEM43). Mutations in TGF3 regulatory domains have been identified in a single large cohort and one individual patient with a clinical diagnosis of AVC. Transforming growth factor B3-modulated desmosomal protein regulation is the presumed pathogenic pathway for this mutation. Mutation and linkage analysis have failed to yield further reports of TGF3-associated AVC. A point mutation in TMEM43 identified in a Newfoundland founder population causes a fully penetrant form of AVC with age- and gender-dependent expression (male predominant), a high burden of SCD, and fibrofatty changes on histopathology. Transmembrane protein 43 is known to respond to proliferator-activated receptor-y signaling, and dysregulation in this pathway may explain the fibrofatty changes seen on histopathology implicating a transdifferentiation pathogenesis. Mutations in RYR2 have been reported with the AVC histological phenotype. The pathophysiological basis for this association is unclear and the clinical phenotype of individuals carrying these mutations is more similar to catecholaminergic ventricular tachycardia (VT) than to AVC.

**Genetic testing in arrhythmogenic cardiomyopathy**

A number of factors limit the use of clinical genetic testing for ACM. A desmosomal variant will be found in around 50% of the patients who fulfil clinical diagnostic criteria, but interpretation may be problematic. Many of these will be single-nucleotide changes that may be found in up to 16% of healthy volunteers. Aside from well-described founder populations, private mutations are common and individually require determination of their pathogenicity as either sufficient to cause disease or sufficient to modify disease in those 10–15% of individuals with AVC who carry more than one variant.

While earlier presentation and more severe phenotypic characteristics have been noted in HCM patients who carry more than one variant, there appears to be a particularly strong relationship between multiple variant carrier status and disease severity in ACM. In a study of 100 families with ACM, more than one variant was present in 28.1% of probands but only 9.7% of relatives (P = 0.01) and the presence of more than one variant was associated with a nearly five-fold increase odds of penetrant disease. These data demonstrate the importance of multiple variants in clinically significant AVC and indicate that sequencing of all five desmosomal genes is required when genetic evaluation of an ACM proband and family is undertaken.

Identification of a pathogenic mutation may enable both detection of pre-clinical disease during which the affected individual may still present with SCD and may also allow for discharge of unaffected relatives from follow-up. Therefore, in cases where clinical diagnosis in the proband is certain or highly probable, genetic testing is reasonable if cascade screening is feasible and desired. However, the data on genetic variants associated with ACM are insufficient to advocate testing in borderline or clinically uncertain cases. For these, clinical follow-up of the proband and his or her first-degree family members is recommended.

**Dilated cardiomyopathy**

Dilated cardiomyopathy (DCM) is characterized by LV enlargement (LVE) and systolic dysfunction. A variety of stressors can cause LV dysfunction including ischaemia, valve disease, hypertension, inflammatory disease, infections, therapeutic cytotoxic medications, and a variety of recreational and performance-enhancing drugs. When DCM occurs in the absence of an identifiable cause, the disease is referred to as idiopathic DCM (IDCM). Systematic non-invasive cardiac evaluation (to include ECG and echo) of first-degree relatives of probands with DCM will identify another affected relative in up to 50% of families. The prevalence of familial DCM (FDCM) by history alone is probably underestimated, as disease expression in family members of clinically apparent probands is often subclinical (mild LVE and minor ECG abnormalities). Accordingly, long-term serial evaluations suggest that DCM is an insidious, slowly progressive inflammatory disease that is familial in the majority of patients. In general, the clinical features of sporadic DCM and FDCM are indistinct with similar ventricular morphologies, non-specific ECG changes,
and the development of clinical HF, atrial and ventricular arrhythmias, stroke, and sudden death seen in both populations. Identifiable phenotypic subsets include DCM with conduction disease often associated with LMNA-encoded lamin mutations and DCM associated with sarcomere mutations that may predispose to earlier disease onset and prominent ventricular arrhythmias.96–99

Since the identification of a mutation in cardiac actin on chromosome 15q14,92 more than 40 genes have been identified in association with non-syndromic FDCM, the majority demonstrating autosomal dominant inheritance.93 Autosomal recessive, X-linked, and mitochondrial patterns account for a minority of cases but represent important clinical subtypes of disease that are discussed in greater detail below.

Familial DCM demonstrates age-dependent penetrance with disease developing in childhood, adolescence, and middle age, but rarely in the elderly.94 Most patients are unaware of the diagnosis until HF symptoms or arrhythmia develops, or abnormalities are detected during routine evaluation. This highlights the important role for active family screening once a proband has been identified. Among the cardiomyopathies, FDCM stands out, in that nearly all disease-causing gene mutations are unique to that family (‘private’ mutations) though examples of variants identified in multiple families exist (TNNT Lys210del; LMNA 203 variants; RNA-binding motif 20—R636).90,95

Familial DCM demonstrates marked genetic heterogeneity with mutations identified in genes encoding sarcomeric proteins (β-myosin heavy chain, cardiac troponin T, troponin C, and tropo- nin I), cardiac muscle LIM protein (CLP), cypher/ZASP (LBD3) and vinculin (VCL), desmoplakin (DSP), desmin (DES), telethonin (TCAP), tafazzin (G4.5), as well as those encoding proteins of the dystrophin-associated complex including ß-sarcoglycan (SCGCD) and dystrophin (DMD).53,89,91,96–106 Disruption of sarcomere–cytoskeletal interactions, myocyte architecture, aggresomal amyloid deposition, desmosomal abnormalities, calcium handling, ion channel function, mitochondrial energy dynamics, and nuclear membrane–cytoskeletal integrity have been described. Because this profound genetic heterogeneity exists in the context of a common phenotype, a final common pathway for disease development has been proposed in which abnormal proteins adversely affect force transmission in the cardiomyocyte leading to cellular injury, inflammation, collagen deposition, remodelling, dilatation, and systolic failure.41,107,108 However, this relatively clean approach to understanding the pathophysiology of FDCM does not allow for ready incorporation of several rather unique and increasingly well-described theories of pathogenesis outlined below.

Desmin is an intermediate filament that forms a scaffold around the Z-disc of the sarcomere and connects the Z-disc to the subsarcolemmal cytoskeleton.109 Mutations in desmin have been demonstrated to lead to pre-amyloid deposition in aggresomal bodies in transgenic mice designed to model desmin-associated cardiomyopathy.110 Interestingly, voluntary exercise reduces deposition of pathogenic aggresomal bodies and dramatically improved survival in this DCM model.111,112

Genes encoding desmosomal proteins, classically associated with AVC, have been recently recognized as a cause of DCM.54,90 The initial report on this topic documented a higher burden of ventricular arrhythmias in DCM patients carrying desmosomal gene mutations. Because this initial report was limited to two families, the familial phenotypic diversity of desmosomal-associated DCM requires further investigation. Isolated reports of AVC masqueraing as DCM have emerged, indicating that considerable phenotypic overlap exists between DCM caused by desmosomal gene mutations, DCM caused by other genetic mechanisms, and AVC.113–115 Clinical presentation with arrhythmia and/or sudden death is more typical of disease caused by a mutation in a desmosomal gene or lamin A/C, where the LV phenotype may have only mild structural and/or functional abnormalities at the time of arrhythmic disease presentations.

Familial DCM with conduction disease secondary to disruption in the nuclear cytoskeleton by mutations in lamin A/C deserves special attention. Nuclear lamins A and C are highly conserved proteins critical in nuclear cytoskeletal integrity. Mutations in these proteins account for 5–8% of FDCM and are particularly notable for the heterogeneity of disease expression with which they are associated. Premature conduction disease, DCM with HF, mild DCM with prominent arrhythmias, partial lipodystrophy, Emery–Dreifuss muscular dystrophy, and Hutchinson–Gilford progeria syndrome are all seen in relation to LMNA mutations.116,117 Lamins A and C play important structural and regulatory roles in the nuclear cytosol. Structural abnormalities of myocyte nuclei associated with LMNA mutations have been observed consistent with the architectural role played by the lamins.118 Abnormally cleaved and processed LMNA-encoded proteins have been associated with diverse phenomena at the nuclear level.119 Reversibility of pathogenic end products and clinical phenotype has been observed in murine models of Hutchinson–Gilford progeria.120–122 The extent to which the underlying disease mechanisms and associated therapies will be applicable to LMNA-associated cardiomyopathy is unclear.

In those affected by LMNA-associated cardiomyopathy, conduction disease can precede development of DCM in some families while in other families DCM occurs first. The practical significance of this is that individuals who may have mild DCM caused by LMNA may be at risk of SCD, while this scenario is highly unlikely with most sarcomere and all cytoskeletal abnormalities. Therefore, when SCD is seen in a family with mild DCM, testing for LMNA mutations may be helpful and lead to early consideration for ICD therapy. Reports of increased arrhythmogenicity in SCN5A-associated and desmosomal-associated DCM indicate that a similar approach may be taken when these mutations are identified.54 Rare variants in sarcomeric genes may be associated with either DCM or HCM depending on the effect of the mutation. Functional analyses of representative sarcomere mutations indicate that disorders of force transmission and of force generation can both lead to development of DCM. Divergent alterations in both calcium regulation (currents and concentration at the level of the sarcolemma) and in thin filament calcium-binding affinity appear to yield different phenotypes with reduced binding affinity favouring development of DCM.88,89,123,124

Dilated cardiomyopathy is also seen as a common component of the muscular dystrophies, including myotonic dystrophy, Friedreich’s ataxia, myofibrillar myopathy, and several limb girdle
muscular dystrophies. Mutations for these disease states are well characterized for the most part and, although outside the breadth of this review, may ultimately provide insight into the pathogenesis of IDCM.

**Utility of genetic testing in dilated cardiomyopathy**

The yield of genetic testing in FDCM is low, \( \sim 30\% \). Given the genetic heterogeneity in DCM, the majority of mutations demonstrate extremely low prevalence necessitating the sequencing of large numbers of genes to enable effective genetic testing. Difficulties in interpreting the results of mutation analysis arise from the high prevalence of private mutations among individual families, and the need to individually assess the pathogenicity of previously unreported mutations that are deemed pathogenic based on structure–function models and evidence of inter-species conservation.

As noted, DCM with conduction disease and/or arrhythmia represents a special subset of FDCM in which focused testing for LMNA, desmosomal, and SCN5A mutations may have a substantial clinical impact. When there is a strong family history of important ventricular arrhythmias, heart block, or SCD, practitioners may consider recommending early prophylactic ICD implantation for genotype-positive relatives, even in the presence of mild or no phenotype.

Identification of a definitively pathogenic mutation in the setting of clinical disease allows for cascade screening which can be a relief for family members who test negative and can then be discharged from follow-up. Likewise, for family members who test positive, appropriate monitoring and interventions can be initiated to prevent disease progression and adverse events. Data supporting the prophylactic use of angiotensin-converting enzyme (ACE)-inhibitors in genotype-positive, phenotype-negative patients with Duchene’s muscular dystrophy for prevention or delay of DCM development are promising,\(^ {127} \) and the use of ACE-inhibitors in asymptomatic LV dysfunction is supported by similar data.\(^ {128} \)

**Other cardiomyopathies**

Restrictive cardiomyopathy (RCM) and LV non-compaction (LVNC) have been subclassified individually but evidence exists for considerable overlap between these syndromes and HCM and DCM. Familial RCM is increasingly recognized as a specific phenotype within the HCM spectrum and can be seen in those who share mutations expressed as classic hypertrophic cardiomyopathy in other family members.\(^ {129,130} \) Similarly, LVNC is an imaging diagnosis with profound overlap with both DCM and HCM phenotypes and their disease-causing mutations.\(^ {131} \)

The prevalence of pure familial vs. sporadic RCM and LVNC (in the absence of HCM and/or DCM within the family) is not known. For LVNC, the definition of the clinical phenotype remains under debate and population prevalence varies widely depending on the cohort examined and the diagnostic criteria utilized.\(^ {132,133} \) Likewise, the clinical course of LVNC remains unclear with some reporting a high incidence of adverse events and others reporting a relatively benign prognosis apparently with the use of the same diagnostic criteria.\(^ {132,134,135} \) Perhaps in part because the clinical syndrome remains under debate, genotypes associated with LVNC span a wide spectrum (cytoskeletal to sarcomeric to ion channel encoding genes).\(^ {99,136–140} \) Genetic testing for LVNC should be reserved for those with syndromic presentation and those with clear familial disease. Because of the overlap with DCM and HCM, active family assessment is essential in evaluation of these patients.

Familial RCM is the rarest of the primary myocardial diseases and is increasingly recognized as an inherited disease seen in association with sarcomere mutations.\(^ {129,141–143} \) The population prevalence remains unknown. Cardinal clinical features include atrial enlargement with normal sized ventricles with a high burden of atrial arrhythmias, progression to advanced HF, and death either related to HF or ventricular arrhythmias.\(^ {144} \) Some RCM-associated troponin I mutations alter troponin I inhibition of actin–myosin ATPase leading to increased calcium sensitivity at the actin–myosin bridge. Increased calcium sensitivity at this site promotes myocardial stiffness by altering sarcomere response to calcium homeostasis. This mechanism may therefore be important in the differential development of RCM vs. HCM phenotypes.\(^ {130} \)

**Modifier genes and environmental effects**

Modifier genes are defined by their effect on expression of primary mutations. The term implies a secondary role in disease development. Modifier genes fail to consistently co-segregate with disease, but when present can significantly alter the phenotypic expression of the primary mutation. Because modifier genes do not co-segregate and are not sufficient to cause disease independently, their identification is a cumbersome task. Nevertheless, because genotype–phenotype plasticity is increasingly appreciated, a growing body of literature has arisen identifying potential modifiers.\(^ {145–160} \)

The impact of diet, fitness, and psychological stress on outcomes in patients with cardiac disease are well documented.\(^ {161–163} \) Among patients with HCM, psychological stress has been found to be a trigger of ventricular arrhythmic events.\(^ {164} \) There are scant but intriguing data that mental stress may impact development of ventricular arrhythmias in AVC.\(^ {165,166} \) Endurance training/sport in patients with AVC is thought to confer increased risk of progression to functional and anatomic abnormalities of the RV and increased risk of arrhythmic death. Risk of SCD in patients with AVC is 5.4-fold higher among competitive athletes than among non-athletes.\(^ {58,167} \)

**Genotype–phenotype plasticity and variance component analysis**

The degree of plasticity in the genotype–phenotype relationship defies explanation by identification of pathogenic rare variants alone. Influences on primary mutation expression include location and type of mutation, number of variants, modifier genes, and
environmental factors. Variance component analysis (VCA) allows comparison of phenotypic variability within and between families carrying the same primary mutation. Segregation of the relative impact of genetic (primary and modifier mutations) and environmental factors may be assessed using this technique. In VCA, the proportion of phenotypic variance that can be attributed to summed genetic effects (primary mutation + modifier genes) is termed heritability. Environmental effects must perforce account for the remainder of phenotypic variability. Figure 3 expresses the relationship between these effects.

Variance component analysis of AVC suggests that modifier genes and environmental effects contribute significantly to phenotypic heterogeneity seen in family members carrying the same mutation, including susceptibility to arrhythmogenesis. Similar analyses have not been systematically undertaken in HCM or DCM cohorts, but the principle maps well onto these diseases and illustrates the complexity of the relationship between pathogenic rare variants and observed phenotype.

A brief note on the drawbacks of genetic testing

This review presents a conservative perspective on the utility of genetic testing. The disadvantages described in the literature mainly focus on the psychological impact of cascade screening, particularly among children and adolescents. Much of the data on the impact of genetic diagnosis and pre/post-test counselling, however, come from the non-cardiac literature. A genetic diagnosis leading to inappropriate device therapy and/or lifestyle restrictions are recognized clinical scenarios. In addition, the financial impact of the broad use of genetic testing is another important factor, though appropriate use of mutation analysis has been shown to be a cost-effective strategy, in that it can free up patients from unnecessary follow-up. In all cases, testing is most useful and least problematic when administered in the context of a multidisciplinary specialty clinic with expertise in the inherited cardiomyopathies.

Conclusions

Clinical and genetic characterization of the inherited cardiomyopathies has lead to novel pathophysiological insights and a new real-time approach to genetic diagnosis. The complexity of genotype–phenotype interaction lends itself to careful clinical observation and judicious use of genetic testing. Caution with regard to application of genetic testing is warranted, in particular with regard to AVC and DCM as interpretation of genetic tests may be limited by phenotypic and genetic heterogeneity as well as prognostic utility. Ongoing efforts to expand our understanding of both pathogenesis of disease and the complex interplay between the factors involved in disease expression will offer continued opportunities for improved care.

Funding

D.J. is supported in part by NIH - 1R21 NR011387 (PI=Redeker) 8/13/09-7/31/11 Cognitive Behavioral Therapy in Stable Heart Failure Role: Co-Investigator. W.J.M.’s work undertaken at UCLH/UCL is supported by a proportion of funding from the Department of Health’s NIHR Biomedical Research Centres funding scheme.

Conflict of interest: none declared.

References


The effects of Wordsworth S, Leal J, Blair E, Legood R, Thomson K, Seller A, Taylor J, 
Tabib A, Loire R, Chalabreysse L, Meyronnet D, Miras A, Malicier D, Thivolet F, 
Hamid MS, Norman M, Quraishi A, Firoozi S, Thaman R, Gimeno JR, Sachdev B, 
Elliott P, O'Mahony C, Syrris P, Evans A, Rivera Sorensen C, Sheppard MN, 
Van Driest SL, Ackerman MJ, Ommen SR, Shakur R, Will ML, Nishimura RA, 
Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, Calkins H, 
Watkins H. DNA testing for hypertrophic cardiomyopathy: a cost-effectiveness 


133. Sandhu R, Finkelhor RS, Gunawardena DR, Bahlert RC. Prevalence and characteristics of left ventricular noncompaction in a community hospital cohort of patients with systolic dysfunction. Echocardiography 2008;25:8–12.


168. Lewis CSH, Jones R. Can we make assumptions about the psychosocial impact of living as a carrier, based on studies assessing the effects of carrier testing? J Genet Couns 2011;20:80–97.
