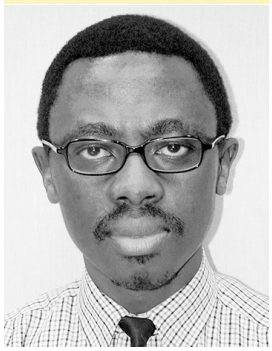
GENETICS AND MOLECULAR DIAGNOSIS OF CARDIOMYOPATHY: WHAT EVERY DOCTOR SHOULD KNOW

The past 15 years has seen major progress towards understanding both the genetic defect and molecular pathogenesis of many monogenic disorders of the cardiovascular system, including hypertrophic cardiomyopathy and familial dilated cardiomyopathy.[1]



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This article reviews the impact of new genetic information on the clinical management of patients and families with hypertrophic cardiomyopathy (HCM) and familial dilated cardiomyopathy (DCM).

WHAT IS KNOWN ABOUT THE GENETIC BASIS OF HYPERTROPHIC CARDIOMYOPATHY?

HCM is characterised macroscopically by left ventricular hypertrophy in the absence of a demonstrable cause (e.g. hypertension, aortic stenosis) and histologically by myocyte and myofibrillar disarray. While it may affect as many as 1 in 500 of the general population, in general medical practice the disease is seen less commonly than DCM.

The vast majority of individuals with HCM have familial disease inherited from a parent as an autosomal dominant trait; true sporadic disease (arising *de novo*) accounts for less than 10% of cases of HCM.[2] Earlier studies suggesting that only 50% of HCM was familial did not recognise that the disease may not be completely penetrant, and that individuals may carry a causal mutation with mild or occasionally undetectable manifestations of HCM. Offspring of such non-penetrant individuals (or 'carriers') are however still at 1 in 2 risk of inheriting the mutation and may well manifest the disease.

Genetic mapping studies in families with HCM have led to the identification of at least 10 disease genes in which mutations can cause the condition (Table I).[1] Mutations are found in the genes for components of the cardiac muscle contractile apparatus, involving both thick and thin filaments of the cardiac sarcomere. Three of these genes appear to be important numerically: β-Myosin heavy-chain, cardiac troponin T, and cardiac myosin-binding protein C account for about two-thirds of genotyped cases. Existing data suggest that each of the three common disease genes is associated with different clinical features and a different prognosis in HCM. Myosin heavy-chain mutations are associated with a wide range of hypertrophic changes. However, available evidence suggests that those mutations associated with a poor prognosis are also associated with overt cardiac hypertrophy and high penetrance. Subclinical carriers with myosin heavy-chain mutations are therefore not likely to be at major risk. By contrast, troponin T mutations typically appear to be associated with the worrying combination of a high incidence of sudden death and mild, often clinically borderline, hypertrophy.[3] Finally, myosin-binding protein C mutations are associated with a late-onset form of HCM that manifests in clinically detectable abnormalities only in mid- to later life; there appears to be a very low incidence of symptoms and sudden death before the onset of hypertrophy in later life.

Experience elsewhere in the world has revealed a variety of mutations in each gene, such that many families have a 'private' mutation (see **http://genetics.med.harvard.edu/~seidman/cg3**). In South Africa, however, there are three recurring (or founder) mutations that have been found in about 45% of genotyped patients of European and mixed ancestry.[4] Consequently, local patients with HCM referred for molecular diagnosis are initially screened for the three founder mutations, and more extensive screening is performed only in their absence.[4]

WHAT IS THE PLACE OF GENETIC TESTING IN PATIENTS WITH IDIOPATHIC DILATED CARDIOMYOPATHY?

True idiopathic DCM is associated with a poor prognosis; therefore the diagnosis should be made after comprehensive investigation to rule out all potential causes, including familial disease. However, 'idiopathic' DCM is sometimes diagnosed without full investigation of the patient to exclude potentially reversible causes of heart failure. Conditions that must be sought on clinical evaluation include hypertension, alcohol abuse, coronary artery disease, valvular heart disease, rheumatic carditis, pericardial disease, cor pulmonale, postpartum and tachycardia-associated cardiomyopathy. An echocardiogram is essential in the evaluation of patients with unexplained cardiomyopathy. Specific blood tests that may be helpful include HIV, thyroid-stimulating hormone, ferritin, and creatine phosphokinase assays.

If no cause is found for the DCM, then investigation by means of cardiac catheterisation and endomyocardial biopsy must be considered. Coronary angiography identifies coronary artery disease in up to 15% of cases of idiopathic DCM; a diagnosis of significant coronary disease would result in a change in the medical options for the patient (e.g. institution of statin therapy) and possibly reveal a surgical option. As a result, we have a low threshold for recommending cardiac catheterisation in our practice to rule out occult coronary artery disease (patients > 40 years) and coronary artery anomalies (younger patients with ischaemic changes on ECG), and to assess suitability for heart transplantation. Endomyocardial biopsy is indicated in selected cases, e.g. those with suspected cardiac haemochromatosis and other infiltrative diseases (such as sarcoidosis) and myocarditis.

The final stage in the evaluation of patients with unexplained DCM is family screening. A careful family history of inherited causes of cardiomyopathy is vital, but the yield is low (identifies 5 - 10% of familial cases). By contrast, screening of first-degree relatives by ECG and echocardiography reveals at least 1 affected family member in up to 30% of cases of unexplained DCM. The identification of asymptomatic individuals with DCM offers the opportunity of treatment with ACE inhibitors and beta-blockers to prevent progression to overt heart failure.

Molecular genetic testing in familial DCM has revealed a far wider range of molecular aetiologies than has been seen in HCM. These include mutations affecting proteins of the cytoskeleton, desmosomal junction, nuclear envelope proteins, sarcoplasmic reticulum, and other regulatory proteins (Table I). In addition, different mutant alleles of the contractile protein genes that underlie HCM are a cause of familial DCM (Table I). The mutations in sarcomeric protein genes may account for up to 10% of cases of childhood-onset familial DCM.

A significant proportion of patients who are given the diagnostic label of 'idiopathic' DCM may have a specific cause, such as familial DCM. The comprehensive investigation of patients with unexplained DCM using the approach outlined above and new molecular methods could result in the disappearance of the diagnosis of 'idiopathic' DCM in our lifetime.

WHAT IS THE IMPACT OF GENETIC INSIGHTS ON CLINICAL MANAGEMENT?

On the basis of the lessons learnt from HCM and DCM families and patients who have been studied at the molecular genetic level in research programmes, some recommendations can be made for the application of molecular genetic insights into the clinical setting. The new genetics has had an impact on:

- better use of the family history
- screening of relatives using clinical tools
- better use of clinical diagnostic tests
- molecular genetic testing in families
- molecular genetic testing in individuals.

Better use of the family history in molecular era

The cornerstone of managing a patient with cardiomyopathy is a full family history of the ages at death and causes of death of close relatives. The risk of sudden death in HCM must be determined by reference to the denominator of presumed affected individuals; in extended families this is a powerful guide to risk. HCM families with characteristic patterns of phenotype-to-risk relationship must be recognised. For example, late-onset HCM with a benign prognosis may point to the presence of a myosin-binding protein C mutation versus patients with a high frequency of sudden death with borderline hypertrophy who may be expected to have troponin T mutations.

When to screen relatives of affected members using clinical tests

First-degree relatives (i.e. parents, siblings and children) of an individual with HCM have a 1:2 risk of being affected, which justifies their screening. By contrast, in DCM cases, screening of first-degree relatives is only justified after exhaustive investigations have been conducted to rule out a reversible cause, as outlined above. In 'true' idiopathic DCM, the prevalence of familial disease is about 30%. Effective treatments are available for the prevention of sudden death in

Table I. **Genes that cause hypertrophic cardiomyopathy and familial dilated cardiomyopathy**

Condition	Gene	Function
Hypertrophic cardiomyopathy	β-Myosin heavy chain α-Myosin heavy chain Myosin essential light chain Myosin regulatory light chain Myosin light peptide kinase Cardiac troponin T Cardiac troponin I Cardiac myosin-binding protein C α-Tropomyosin Cardiac actin Titin	Contractile proteins of the sarcomere
Familial dilated cardiomyopathy	β-Myosin heavy chain Cardiac myosin-binding protein C Cardiac troponin T α-Tropomyosin Cardiac actin Titin Telethonin	Contactile proteins of the sarcomere
	Phospholamban	Regulator of cardiac muscle sarcoplasmic reticulum Ca(2+)-ATPase
	Dystrophin Desmin δ-sarcoglycan Metavinculin Muscle LIM protein	Cytoskeletal proteins
	Lamin A/C Emerin	Inner nuclear membrane proteins
	Tafazzin	Enzyme that produces glycophospholipid of the inner mitochondrial membrane
	Desmoplakin	Desmosomal junction
	ATP-binding cassette, sub-family C, member 9	Regulatory sulfonylurea receptor 2A (SUR2A) subunit of the cardiac K(ATP) channel

HCM (e.g. implantable cardioverter defibrillator), and for retarding progression to heart failure in asymptomatic DCM (e.g. ACE inhibitors). Clinical screening by history, physical examination, ECG and echocardiography should therefore be offered to first-degree relatives of cases of HCM and unexplained DCM. Screening should always be coupled with genetic counselling because of the complexity of preclinical diagnosis. The wishes of family members who do not want to participate must be respected.

Clinical screening in individuals at risk of HCM is best performed every second year in childhood and annually throughout adolescence. In families who present with disease in adolescence or in early adult life, screening can usually be discontinued when they are in their early 20s. In individuals diagnosed later in life, there is a possibility of late-onset HCM, so a normal clinical examination in their 20s does not exclude the later development of the disease and occasional further review (especially in the advent of symptoms) is appropriate.

Better use of clinical diagnostic tests

With the availability of molecular diagnosis as a gold standard, it has been possible to re-evaluate the sensitivity and specificity of clinical diagnostic criteria. In HCM and familial DCM, it has become clear that many affected individuals do not have the classic features of the disease, and subtle signs must be interpreted in the context of the high prior likelihood of disease. Different diagnostic criteria must be used in families with HCM and familial DCM.[5,6] It is important not to overlook the following:

- ECG abnormalities (e.g. left axis deviation), even in the presence of a normal echocardiogram. For example, in HCM it is recognised that the ECG becomes abnormal before the echocardiogram shows hypertrophy, especially in children.[5]
- Borderline hypertrophy, e.g.13 mm maximal wall thickness in suspected HCM.
- Systolic (Sa) and diastolic (Ea) myocardial velocities measured by tissue Doppler imaging (TDI) were shown recently to be decreased in subjects who have mutations causing HCM but who do not have left ventricular hypertrophy. TDI predicts the later development of HCM in individuals with myocardial velocity abnormalities.

- Isolated left ventricular enlargement with preserved systolic function must be considered suspicious in a family member with a relative affected with idiopathic DCM.

When is molecular genetic testing indicated in families?

Clinical tools will detect only a proportion of gene carriers in affected families. If there are 3 or more clinically affected individuals in a family with genetic disease in whom the disease- causing genes are known, it is usually possible to use linkage analysis to determine which disease gene is likely to be mutated and which therefore should be screened.

A molecular genetic diagnosis may be useful in families with HCM and familial DCM in situations in which clinical diagnosis is not possible and in which the diagnosis will change the management of the patient, e.g. HCM or familial DCM in families with an extremely high incidence of sudden death or heart failure in young individuals. Molecular genetic testing may be useful for antenatal diagnosis or diagnosis in children and adolescents in these families.

The clinical diagnosis of HCM is one of exclusion, so a firm diagnosis can sometimes not be reached in individuals who are hypertensive, markedly obese, or highly athletic. If such individuals are members of an extended family, a molecular genetic diagnosis can be sought if the presence of a disease-causing mutation will change the management of the patient (e.g. in families with a 'malignant' mutation). This information will be important — not just for their management but also to determine whether their first-degree relatives are also at risk.

When is molecular genetic testing indicated in individuals?

Mutation detection in single affected individuals, which is often requested by physicians who are presented with an uncertain diagnosis, is technically demanding, and in most cases the work involved will not be justified by its clinical usefulness. In HCM, molecular genetic testing in single individuals is probably appropriate only where relatives of a surviving patient died suddenly and were found to have minimal hypertrophy (i.e. troponin T-type phenotype) — a genetic diagnosis would then be of relevance to all family members. In survivors of an HCM case diagnosed at autopsy, and in the absence of living affected individuals, genetic screening is currently not useful because failure to find a mutation does not exclude the diagnosis. A useful approach is to screen all first-degree relatives clinically.

Outside research protocols there is currently no place for molecular genetic screening of single individuals with idiopathic DCM with no history of familial disease.

WHERE TO REFER PATIENTS FOR MOLECULAR GENETIC SCREENING OF INHERITED CARDIOMYOPATHY IN SOUTH AFRICA

Currently the work is undertaken by two research laboratories with a specialist interest in cardiovascular disorders:

- Hypertrophic cardiomyopathy gene testing — Dr Hanlie Moolman-Smook, Stellenbosch University/ MRC Centre for Molecular and Cellular Biology, Faculty of Health Sciences, Stellenbosch University, Tygerberg, tel (021) 938-9406, fax (021) 938-9476, e-mail: **Hm@sun.ac.za**
- Dilated cardiomyopathy gene testing — Dr Bongani M Mayosi, The Cardiac Clinic and Division of Human Genetics, Departments of Medicine and Clinical Laboratory Sciences, Groote Schuur Hospital and University of Cape Town, tel (021) 404-6064, fax (021) 448-7062, e-mail: **bmayosi@uct gsh1.uct.ac.za**

References available on request.

Further reading

Fatkin D, Graham RM. Molecular mechanisms of inherited cardiomyopathies. *Physiol Rev* 2002; **82:** 945-980.

Maron BJ, McKenna WJ, Danielson GK, *et al.* American College of Cardiology/ European Society of Cardiology Clinical Expert Consensus Document on Hypertrophic Cardiomyopathy: A Report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents and the European Society of Cardiology Committee for Practice Guidelines. *J Am Coll Cardiol* 2003; **42:**1687-1713.

IN A NUTSHELL

A complete and detailed family history is the cornerstone to the management of patients with cardiomyopathy.

Idiopathic DCM is a diagnosis of exclusion that should only be made after exhaustive non-invasive and invasive investigation to rule out potentially reversible causes of heart failure.

Clinical screening should be offered to all first-degree relatives (i.e. parents, siblings and children) of cases of HCM and idiopathic DCM.

A much lower diagnostic threshold is appropriate when interpreting diagnostic tests in first-degree relatives of patients affected with HCM and familial DCM. In particular, there should be careful examination of the ECG and the echocardiogram for subtle abnormalities in these cases.

A molecular genetic diagnosis can be useful in HCM in settings in which the clinical diagnosis is equivocal (e.g. borderline hypertrophy) or impossible (e.g. in the presence of hypertension) in association with a high incidence of sudden death, but this remains technically demanding.

Molecular genetic diagnosis in isolated individuals is generally not practical.

Laboratories that offer molecular genetic testing are limited to research institutions with an interest in inherited cardiovascular disorders. (Information about South African laboratories is supplied in the text.)