Hypertrophic cardiomyopathy (HCM) is a myocardial disorder characterized by left ventricular (LV) hypertrophy without dilatation and without apparent cause (ie, it occurs in the absence of severe hypertension, aortic stenosis, or other cardiac or systemic diseases that might cause LV hypertrophy). Numerous excellent reviews and consensus documents provide a wealth of additional background.1–8 HCM is the leading cause of sudden death in young people and leads to significant disability in survivors. It is caused by mutations in genes that encode components of the sarcomere. Cardiomyocyte and cardiac hypertrophy, myocyte disarray, interstitial and replacement fibrosis, and dysplastic intramyocardial arterioles characterize the pathology of HCM. Clinical manifestations include impaired diastolic function, heart failure, tachyarrhythmia (both atrial and ventricular), and sudden death. At present, there is a lack of understanding of how the mutations in genes encoding sarcomere proteins lead to the phenotypes described above. Current therapeutic approaches have focused on the prevention of sudden death, with implantable cardioverter defibrillator placement in high-risk patients. But medical therapies have largely focused on alleviating symptoms of the disease, not on altering its natural history. The present Working Group of the National Heart, Lung, and Blood Institute brought together clinical, translational, and basic scientists with the overarching goal of identifying novel strategies to prevent the phenotypic expression of disease. Herein, we identify research initiatives that we hope will lead to novel therapeutic approaches for patients with HCM.

Epidemiology

The epidemiology of HCM suggests that it is present in ≈1 in 500 adults.9 Because of the delay in phenotypic expression of the disease, HCM is not commonly recognized clinically in young children, but when it is, it is much more frequently recognized in males.10 This is likely due to greater penetrance in young males.11,12 HCM is underdiagnosed clinically in blacks and in women, yet women tend to present with more marked heart failure than men when they are diagnosed later in life.13,14 There is no overall difference in mortality, including sudden cardiac death, between men and women, although sudden cardiac death on the athletic field predominantly occurs in men.

 Genetic Cause

Extensive investigation has shown that at least 50% of HCM cases can be traced to a specific genetic cause. This probably underestimates the true percentage of genetically based HCM, because current mutation-screening platforms typically examine only 8 to 10 genes because of unfavorable cost-benefit assessments. For example, current platforms do not examine titin (owing to its size) or myozeenin-2 (because relatively few mutations have been defined in this gene).15

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Moreover, when strict clinical criteria are used for diagnosis, including family history, mutation detection approaches 70%.

HCM is a genetic disease of sarcomere proteins,\(^{5,16}\) with mutations in the genes that encode β-myosin heavy chain (MYH7) and myosin binding protein C (MYBPC3) accounting for \(\approx 80\%\) to 85% of cases with identified mutations in most series.\(^{4,17–19}\) Mutations in the troponins cardiac troponin T (TNNT2) and troponin I (TNNI3) and α-tropomyosin (TPM1) are also relatively common, collectively representing 10% to 15% of additional genetic causes for all HCM cases.\(^{4,17–19}\) These and the myosin light chains (MYL2, MYL3) and α-cardiac actin (ACTC) are the 8 genes most commonly involved in HCM.

### Rare Genetic Causes

Mutations in several other genes that encode sarcomere or sarcomere-related proteins have been implicated in HCM, including cardiac troponin C (TNNC1), α-myosin heavy chain (MYH6), and cardiac myosin light chain 2 (MYLK2). In addition, several other genes that encode non-sarcomere proteins, including caveolin 3 (CAV3), calreticulin (CALR3), junctophilin-2 (JPH2), phospholamban (PLN), and the mitochondrial tRNA-encoding genes MTTG and MTTI, produce clinical features that mimic HCM.\(^{4,20}\) The relationship of HCM caused by sarcomere protein gene mutations to these disorders is unclear.\(^{5,16}\)

### Unknown Causes

The apparent absence of mutations in patients with a clinical diagnosis of HCM indicates an important gap in our knowledge. Mutation testing can be particularly uninformative in 3 clinical scenarios in which family history is negative: (1) Hypertrophy that occurs very early in childhood; (2) hypertrophy that is only recognized after middle age; and (3) hypertrophy that is limited to the ventricular apex.\(^{21}\) The cause of these conditions is not clear.

### Disease Mechanisms

The disease mechanisms of HCM remain incompletely understood. Postulated mechanisms include (1) a dominant negative function (ie, a “poison peptide,” wherein the mutant gene encodes a protein that interferes with the function of the normal allele); (2) haploinsufficiency (leading to an insufficient quantity of the normally functioning sarcomere protein); and/or (3) impaired myocardial energetics and decreased energy reserve.\(^{8,22,23}\) The lack of a definitive link between mutations and an understanding of the pathogenesis/molecular mechanisms that drive the expression of the HCM phenotype is a significant gap in our understanding of the disease.

### Clinical Genetics

Allelic heterogeneity (each family having a so-called private mutation) is particularly common in HCM. Approximately 500 mutations have been noted in the medical literature, but the number of identified mutations in various private databases suggests the true number is more than 1000. HCM demonstrates age-dependent penetrance, affecting 50% to 80% and 95% of individuals by age 30 years and ages 50 to 60 years, respectively.\(^{24}\) Recent estimates of 1% annual mortality in HCM differ significantly from earlier estimates (3% to 6%) that were based on referral populations of high-risk groups to HCM centers.\(^{25–27}\) Survival to 75 years or beyond has been estimated in \(\approx 25\%\) of an unselected HCM cohort.\(^{28}\)

### Compound Heterozygosity

Two to five percent of patients with HCM harbor 2 mutations (compound or double heterozygosity) or are homozygous for a mutation.\(^{4,18,29,30}\) and these patients display more severe and/or earlier onset of disease.\(^{29,30}\)

### Genotype/Phenotype Relationships

Despite the significant clinical heterogeneity observed even for the same mutation within families or between families,\(^{31–35}\) as well as the variable penetrance, which alters clinical onset and severity of disease, genotype/phenotype relationships of sarcomere gene mutations clearly have advanced our understanding of the disease and, in some cases, have allowed identification of relatively low- versus high-risk patients.\(^{36,37}\) Some genotype/phenotype relationships that have stood the test of time include MYH7 mutations, which are associated with earlier onset and more extensive hypertrophy.\(^{8,33,35}\) More specifically, the myosin 403 mutation is associated with increased risk of heart failure and sudden death, and the myosin 719 mutation leads to a marked increase in heart failure. Others include the relatively limited hypertrophic response with TNNT2 mutations\(^{34,38,39}\) and the incomplete penetrance and relatively later onset of HCM from MYBPC3 mutations.\(^{31,36,40}\) That said, multiple poorly understood mechanisms contribute to heterogeneity of presentation, and these include environmental inputs, sex,\(^{10}\) and genetic and epigenetic modifiers.

### Key Morphological and Clinical Components of Genetically Mediated HCM

Cardiomyocyte and cardiac hypertrophy, myocyte disarray, interstitial and replacement fibrosis, and dysplastic intramyocardial arteries characterize the pathology of HCM. Clinical manifestations include impaired diastolic function, tachyarrhythmia (both atrial and ventricular), and sudden death.\(^{41–44}\) Accepted risk factors for sudden cardiac death include prior cardiac arrest from ventricular fibrillation, spontaneous sustained ventricular tachycardia, family history of premature sudden death, unexplained syncope, LV wall thickness \(\geq 30\) mm, abnormal blood pressure response to exercise, and nonsustained ventricular tachycardia.\(^{28,43}\) Additional predictors include the presence of LV apical aneurysms and the end stage of disease.\(^{45,46}\)

A consensus document\(^1\) and review\(^{37}\) of clinical management of arrhythmias and sudden cardiac death in HCM are available. Clinically apparent atrial fibrillation develops in at least 20% of patients with HCM,\(^{48}\) but the true incidence is likely higher than that. Atrial fibrillation is a risk factor for thromboembolic disease, including stroke.\(^1\) Molecular mechanisms that regulate the phenotypic expression of the various pathologies and how these might drive arrhythmogenesis in HCM are poorly understood.

### Genetic Causes of LV Hypertrophy Not Involving Sarcomere Mutations

LV hypertrophy can result from gene mutations that alter proteins with functions that are unrelated to the sarcomere. These
include Fabry disease, glycogen storage disorders (PRKAG2 cardiomyopathy and Pompe disease), lysosomal disorders (X-linked lysosome-associated membrane protein gene cardiomyopathy), and several syndromes (ie, Noonan, lentigines, ECG abnormalities, ocular hypertelorism, pulmonary valve stenosis, abnormal genitalia, retardation of growth, and deafness [LEOPARD], and Costello). The clinical manifestations and patient courses associated with these are different from HCM.8,17,49–51 These phenocopies are not discussed further herein.

**Rationale for Investing in Research on HCM**

The rationale for investing in research in HCM is supported by the following: (1) HCM is the most common genetic heart disease and affects individuals at every age; (2) HCM is the most common cause of sudden death in young people; (3) HCM is an important cause of heart failure disability; (4) HCM can be viewed as a paradigm for the potential opportunities provided by harnessing modern genetic science in medicine to make gene-based diagnosis and prediction a reality; (5) gaps in our basic understanding of mechanisms of disease are substantial, but already, insights provided by studies in patients and in animal models of HCM suggest creative strategies to alter the natural history of this disease; and (6) these insights also promise a greater understanding of the molecular pathophysiology of other, nongenetic causes of hypertrophy. Thus, we believe that there are unparalleled opportunities in the immediate and near future to translate basic insights about HCM into new clinical models for diagnosis, prevention, and therapy.

**Critical Deficits in Our Understanding of HCM Pathogenesis Define Research Initiatives**

In the online-only Data Supplement to this report, we define key deficits in our understanding of HCM, thereby leading into a delineation of research initiatives for the future. The areas of research will be broken down into clinical, translational, and basic science sections, but these divisions are clearly arbitrary and only serve as an organizational (and not operational) tool. In fact, we will strive to maintain connections among the 3 divisions, focusing on common deficits in our understanding.

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Research Priorities in Hypertrophic Cardiomyopathy: Report of a Working Group of the National Heart, Lung, and Blood Institute


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Supplemental Material

Research Priorities in Hypertrophic Cardiomyopathy:
Report of a Working Group of the National Heart Lung and Blood Institute
I. Clinical Initiatives.

A. There is an incomplete understanding of the genetics of HCM.

Recommendation: Define all genetic causes for HCM.

Current strategies define pathogenic mutations in only a subset of HCM patients. Whether technical issues related to mutation analysis platforms and/or undiscovered genes account for this is unknown. Broad-based application of contemporary robust sequencing platforms to large populations of patients clinically diagnosed with HCM can answer this question. Knowledge of the full spectrum of genes and mutations in HCM is important to explain pathogenesis and to interrogate clinical manifestations.

The increasing application of gene-based diagnostics in HCM has led to the recognition of patients in whom a disease-causing mutation is not identified. Some of these patients with classic clinical manifestations of HCM have a positive family history, a scenario that confirms that current gene-based diagnostics are incomplete. Failure to define the genetic cause for familial HCM may reflect undiscovered disease genes or incomplete analyses of known disease genes. As noted above, current mutation screening platforms typically examine 8-10 genes that encode sarcomere proteins in which many HCM mutations have been reported, but several other identified HCM disease genes are rarely examined (e.g., titin and myozenin-2). Moreover, contemporary genetic analysis platforms that employ traditional sequencing strategies can survey nucleotide variation but fail to assess structural variations in these genes (e.g., insertions, deletions, and variation in copy number of a gene). With emerging data confirming structural variation in some HCM genes, particularly MYBPC3, it is timely to consider the application of newer sequencing technologies in gene-based diagnosis of HCM. These technologies have the potential to efficiently fully interrogate the spectrum of human mutations including nucleotide and structural variations and in all disease genes, though at present, cost remains a significant impediment. That said, the benefits of improved accuracy of gene-based diagnosis in HCM is particularly relevant for accurate identification of at-risk family members. Identification of mutation-carriers without clinically overt HCM (i.e., pre-clinical HCM) is important to fully define disease pathogenesis in HCM. Study of this population may help elucidate factors that contribute to or prevent disease
development. Broad-based genetic diagnosis can also foster the identification of “mutation-negative” HCM families. Study of these could lead to the discovery of new HCM genes.

As indicated above, mutation analyses are often uninformative in HCM patients who lack a family history of disease, a scenario that is not uncommon in young children and older adults who present with unexplained hypertrophy. These particular cohorts raise multiple questions for research and clinical medicine. Do these individuals have a genetic or an acquired disorder? Are there monogenic causes of HCM that reflect recessive or X-linked mutations? Can somatic mutations in primordial cardiomyocytes cause HCM? Can complex genetics (variation of multiple risk genes) cause HCM? And for each of these different genetic models, is the pathology sufficiently related to HCM by both pathophysiological mechanisms and clinical course to warrant a diagnosis of HCM or another label?4,5

Application of contemporary genetic and genomic strategies will help to answer these questions. Comprehensive sequence analyses of myocardial tissues can assess the role of somatic mutation in HCM. Developing cohorts of mutation-negative HCM patients can direct family-based and population-based genome-wide studies to define new genes and risk alleles.6 Early clinical studies of mutation-positive versus mutation-negative HCM have begun to suggest differences in morphologic manifestations and clinical outcomes.4 By defining the cause of disease in mutation-negative HCM, there is an opportunity to discover cell and molecular responses triggered by these genetic etiologies, and the potential for gene-specific therapeutics. More broadly, this knowledge base will provide a more comprehensive understanding of molecular triggers for cardiac remodeling, data that may be relevant to a wide range of cardiac pathologies.

B. The natural history of HCM should be more clearly defined.
Recommendation: Support a prospective study of the natural history of HCM.
Large referral centers have utilized their own referral populations to define the natural history of HCM, including identification of risk factors for progression to heart failure and for sudden cardiac death.7-12 These studies provided important insights and constitute much of the basis for current treatment guidelines for patients with HCM and
for their family members.\textsuperscript{12} That said, much of the data provided by these studies has one or more limitations including relatively small sample sizes, center-specific differences in diagnostic criteria for HCM, and differences in practice patterns and patient follow-up. Importantly the definition of HCM utilized by many cohort studies are based on clinical criteria and fail to capitalize on genetic information that precisely define patients with the same or different etiology.

In the past, studies of the natural history of HCM could not take advantage of emerging imaging techniques (e.g. MRI), new strategies to assess arrhythmic risks (e.g. circadian rhythm patterns or long-term arrhythmia monitoring with implantable devices) or newer biomarkers\textsuperscript{13–21}. Although some of these approaches are being studied in selective centers, results may reflect referral populations, similar to earlier clinical studies. We believe that new tools are now in place to establish a multi-center prospective observational cohort study of HCM that is predicated on genotype.\textsuperscript{22} Application of the same comprehensive sequencing strategies to define pathogenic mutations in HCM patients described above will provide clinical researchers with a rigorous framework for detailed phenotypic characterization. For patients with pathogenic mutations, a more complete delineation of the natural history of HCM is now feasible, in that gene-based diagnostics can identify mutation carriers who have not yet developed clinical disease. By employing advanced cardiac imaging, contemporary arrhythmia interrogation platforms, and serum biomarkers to interrogate the pre-hypertrophic phase of HCM, information about the earliest changes in myocardial structure, function, physiology and even biochemistry will be obtained. By defining clinical manifestations of emerging disease, investigators can assess the roles of intrinsic biological triggers (e.g., sex, growth, hormones) and environmental modifiers/lifestyle (physical activities, diet, blood pressure, etc.) that may promote/retard disease development. Improved knowledge of the features of pre-hypertrophic manifestations of disease are important to develop surrogate endpoints that can be used to assess therapeutic interventions aimed at preventing phenotypic expression of the disease.

The primary goals of this study should be to 1) define clinical manifestations of HCM across a range of mutations; 2) fully characterize the pre-clinical stage of HCM; 3) refine diagnostic criteria using both clinical and genetic data to distinguish HCM due to
mutations of genes encoding sarcomere proteins from disorders that are clinical phenocopies (e.g., storage disorders, hypertensive LVH); 4) develop sufficient sample sizes to classify HCM patients into groups with similar genetic lesions and compare their natural history and the natural histories of their family members, starting prior to the onset of phenotypic disease; 5) collect serial blood specimens to foster development and testing of novel circulating biomarkers that may track disease progression and response to therapy; 6) perform serial echocardiograms, with central core laboratory analysis, to clarify the natural history of cardiac remodeling in relation to patient genotype, and 7) identify common genetic variants and exposures that modify expression of the HCM phenotype. More focused studies in selected subgroups could include studies using advanced imaging techniques such as cardiac magnetic resonance (CMR), $^{31}$P-nuclear magnetic resonance (NMR) spectroscopy, and/or positron emission tomography (PET), and potentially even endomyocardial biopsies to provide tissue for translational studies (see below).

Recognizing that there will be a trade-off between sample size and the number and complexity of assessments performed on each subject, this study should emphasize sample size over complexity so that sufficient power will be available to study genetic subgroups. This research is expected to yield substantial and generalized insights into the diagnosis and progression of HCM that will refine clinical practice. In addition, it will provide a substrate for translational studies of biomarkers and possibly cardiac tissue and aid in design of clinical trials by identifying subgroups of HCM patients that may benefit the most from specific treatment strategies.

C. There is a paucity of evidence-based treatment strategies for HCM patients. Recommendation: When potential therapeutic strategies are identified in pre-clinical studies and early clinical trials, support a randomized, placebo-controlled multi-center clinical trial.

Although we fully realize that the state of current knowledge does not allow us to identify a specific intervention to test at this time, this section will outline the rationale for, and potential design of, a theoretical clinical trial, including possible surrogate
endpoints and pitfalls, should a candidate intervention be identified in pre-clinical studies (see below) and supported in early phase clinical trials.

**Current therapeutic approaches to HCM.**

The medical management of HCM has been largely unchanged over the past decades\(^2\) and in contrast to therapies for ischemic heart failure, treatment recommendations in HCM are based on observational series without prospective randomized controls. Contemporary approaches to management are discussed more fully in the following references.\(^{12,24,25}\) While clinical usage provides support that various pharmacologic agents reduce HCM symptoms, no evidence has demonstrated that they alter disease progression or outcomes. With the discovery of causal gene mutations and the development of animal models that allow interrogation of pathogenic mechanisms, research is needed to consider newer strategies in HCM. With the ability to identify individuals with HCM mutations in advance of clinical disease, opportunities should be explored to find therapies that impact the natural history of HCM by preventing, or attenuating the emergence of myocardial phenotypes.

A major goal in treatment of HCM is to limit the life-threatening consequences of arrhythmia. As precise triggers for arrhythmias that produce SCD in HCM are unknown, management strategies aim to treat high risk patients (defined by factors detailed above) with implantable cardiac defibrillators (ICD). While these devices can avert SCD and confer a favorable risk-benefit ratio,\(^2\) more robust parameters that discriminate between HCM patients who will vs. will not benefit from ICD implantation are needed. Furthermore, while no anti-arrhythmic agents have been shown to prevent SCD, novel agents may emerge, and their efficacy could be assessed in high risk patients with ICDs already in place.

For patients with symptoms related to left ventricular (LV) outflow obstruction, current therapies aim to diminish the mechanical obstruction.\(^{23,25}\) When symptoms are refractory to medical therapy, surgical septal myectomy is warranted.\(^{27-30}\) The development of catheter-based alcohol septal ablation, can benefit select patients, although long-term experience with this procedure is limited and its appropriate use continues to be debated.\(^{27-30}\) Well-controlled trials involving these treatment options
would add substantially to clinical management of HCM.

AF may be poorly tolerated in patients with HCM, and rate control or measures to convert to sinus rhythm are important aspects of therapy. In patients with a history of AF, chronic anticoagulation is essential. Knowledge of why AF is substantially increased in HCM and strategies to prevent this arrhythmia would benefit large numbers of HCM patients.

**Potential drug therapies and novel targets for intervention**

At present, none of the routinely employed pharmacologic therapies for symptomatic patients with HCM have been demonstrated to impact beneficially on the natural history of HCM. Furthermore, it is unclear whether any currently available drug therapy can prevent (or limit) the development of phenotypic manifestations of HCM in individuals identified as carrying a known disease-causing sarcomere mutation. That said, some preclinical evidence supports the potential benefit of drugs that block the renin-angiotensin-aldosterone system (RAAS). In patients with HCM, serum levels of aldosterone are increased up to 4-fold compared to normal controls, a finding which is accompanied by increased levels of serum markers of collagen turnover and abnormalities of diastolic function. In addition, several recent studies using transgenic mouse models of HCM have demonstrated up-regulation of the RAAS. Spironolactone has been shown to reduce both coronary microvascular remodeling and fibrosis and to improve diastolic function in mouse models of HCM expressing human sarcomere mutations. However, similar strategies in the feline HCM model have not shown similar benefit.

Four small pilot studies in patients with non-obstructive HCM have reported that angiotensin receptor antagonists led to borderline improvement in various parameters. Most recently, Penicka et al. have reported benefits with candesartan (reduced LVH and improved exercise tolerance). They also reported that treatment effects may be dependent on the specific sarcomere mutations, although this, as with all clinical trials to date in HCM, was too small to draw any definitive conclusions. At present, it is not clear whether targeting the RAAS in HCM will be beneficial in patients, but at least the studies suggest the angiotensin receptor antagonists are safe in non-obstructive disease.
Studies in mouse models suggest that other strategies might be effective. For example, the L-type calcium channel inhibitor, diltiazem, has had beneficial effects in two HCM models- the α-myosin heavy chain(403/+) and TnT 179N mutations. Diltiazem is currently being examined in patients with documented HCM mutations but with no evidence of phenotypic expression of disease to determine whether it can prevent clinical manifestations (http://clinicaltrials.gov; trial # NCT00319982). Data from animal models also support a potential role of statins though a recent pilot study in patients showed no effect on hypertrophy or contractile function. Emerging evidence suggests that targeting BMP and TGFβ signaling in the heart may hold promise as well, particularly for blocking the fibrotic response (see Basic studies, section D). In fact, RAAS inhibitors likely act, in part, by inhibiting TGFβ. However, much more pre-clinical work needs to be done with this system.

Clearly, there is a critical need for future research considerations directed toward basic and translational studies to identify putative targets for intervention (see below). When viable targets are identified, clinical studies should be undertaken to determine if novel (or currently available) drug, or other therapies, can target these pathways of HCM disease expression and, thereby, improve on the natural history of patients with this disease.

**Challenges for clinical trials in HCM.**

There are important obstacles to prospective, randomized clinical trials in patients with HCM, most notably the low overall event rate. For sufficient enrollment, participation by multiple centers would be needed. Furthermore many “hard” or objective outcomes in HCM, occur after many years (e.g. heart failure) or are uncommon (e.g. sudden cardiac death, for which the estimated event rate is ~1% per year). Thus prospective trials to assess the efficacy of new drugs or devices to reduce these HCM outcomes would require enrollment of large patient numbers studied for many years. Furthermore, genetic and phenotypic diversity in HCM would make individual patient randomization even more challenging. Despite these challenges, prospective trials that use surrogate end-points in HCM may be feasible.
Efforts to assess existing or novel drugs or devices in HCM require: 1) identifying pathophysiologic pathways of HCM disease expression that are responsible for adverse myocardial remodeling and unfavorable disease consequences; 2) documenting clear linkage between clinical outcomes and biomarkers, including imaging end-points, that reflect disease pathophysiology or progression, thereby identifying credible surrogate end-points for clinical trials; 3) accumulating sufficient evidence to support the use of existing or novel drug therapy to intervene on these pathways in an attempt to beneficially alter myocardial structure and function; and 4) assuring that results can be obtained within a reasonable study duration.

**Potential pathophysiologic targets and surrogate endpoints in HCM.**

Among the potential surrogate endpoints in HCM, one focus could be on myocardial dysfunction capitalizing on newer imaging modalities (i.e., MRI, tissue doppler imaging and strain). Alternative endpoints with probably more promise, given apparent linkage to clinical outcomes, include measures of myocardial ischemia, myocardial hypertrophy (LV mass), and fibrosis. The severity of abnormalities in these endpoints may be more closely linked to the adverse clinical course, including a propensity to worse symptoms, arrhythmias and progressive myocardial dysfunction.

In HCM, the mechanism of myocardial ischemia is likely related to structurally abnormal intramural coronary arterioles, resulting in microvascular dysfunction, reduced vasodilator reserve, limiting myocardial blood flow (MBF) during stress. Previous studies that employed contemporary cardiovascular imaging modalities such as single photon emission tomography (SPECT), positron emission tomography (PET) and cardiovascular magnetic resonance imaging (CMR), have demonstrated that HCM patients have impaired MBF following dipyridamole administration. Impaired MBF is a powerful independent predictor of adverse LV remodeling and of cardiovascular mortality. Among these imaging modalities, CMR may be particularly advantageous for patients with HCM by providing, in one examination, detailed characterization of the pattern and distribution of wall thickening, as well as an assessment of myocardial perfusion and fibrosis, with high spatial resolution and without ionizing radiation. With intravenous gadolinium administration, CMR perfusion sequences permit both qualitative and
quantitative assessment of MBF at rest and during pharmacologic stress with superior spatial resolution compared to PET or SPECT. Furthermore, after the acquisition of the first-pass perfusion images, late gadolinium enhancement sequences (LGE-CMR) can identify the presence (and quantitatively measure the extent) of myocardial fibrosis in HCM patients. LGE may be one surrogate endpoint since HCM patients with the most marked reduction in ejection fraction have more extensive LGE than patients with preserved systolic function. Holter monitoring shows ventricular arrhythmias are also more common in HCM patients with LGE than HCM patients without LGE. Whether CMR can delineate an unstable arrhythmogenic substrate and identify patients at risk for sudden cardiac death warrants study.

PET and CMR studies in HCM patients demonstrated blunted MBF in response to stress. Moreover HCM myocardium that is near to, or contains regions with LGE are often associated with reduced MBF by PET and CMR. Taken together, these observations suggest that myocardial ischemia may contribute to myocyte death and ultimately replacement fibrosis. These data support efforts to determine if myocardial ischemia precedes LGE-labeled myocardium in HCM, and assessment of whether strategies to mitigate ischemia might prevent myocyte death and the emergence of replacement fibrosis in HCM. Ischemia and fibrosis also represent potential pathophysiologic targets for therapeutic intervention using existing or novel drug therapy. Based on these findings, imaging markers of myocardial ischemia, along with those for pathologic hypertrophy and fibrosis, represent prime candidates to serve as surrogate endpoints for clinical investigation into the role of various prevention and treatment strategies.

Construct of a potential clinical trial in HCM.

A clinical trial aimed at determining the efficacy of drug therapy in altering phenotypic expression should identify HCM patients during their pre-clinical or early clinical phase of disease expression. This will allow a focus on prevention of progression of disease. Identifying patients based on genotype at this phenotypically early stage would permit assessment of the prevalence and extent of ischemia and fibrosis, prior to development of more obvious adverse remodeling. It would therefore help to determine
the potential to favorably alter disease expression. Most likely patients would be identified in adolescence or early adulthood. Patients would likely be best identified by cascade evaluation of family members with overt HCM.

For an initial clinical trial, enrollment should target patients with mutations that are more common, and that are characterized by a more robust phenotype that more predictably progresses over a shorter period of time (e.g. MYH7). Patients with minimal evidence of disease by echocardiography would seem the most logical to target, but consideration might also be given to enriching the population by targeting a percentage of patients enrolled with early phenotypic findings.

The primary endpoint of an initial clinical investigation should be a marker with biologic plausibility for linkage to clinical natural history and that maps closely with clinical outcomes. As discussed above, candidate primary endpoints include LV mass and evidence of ischemia or fibrosis on CMR. Clinical outcomes should be tracked as well, but it will be difficult or impossible to power an initial study for clinical outcomes, based on the low event rates in this population. Nevertheless, strong consideration should be given to incorporating specific clinical events, such as death or life-threatening arrhythmia, into a composite endpoint, along with a disease marker such as progression to LVH. However, because LVH and fibrosis are relatively late markers of disease, multiple potential biomarkers of disease progression should also be examined. These should include serum markers of fibrosis and collagen turnover (e.g. the pro-sclerotic cytokines and the protease/anti-protease systems, TGFβ1, IGF-1) and markers of myocyte stretch, hypertrophy, and injury (e.g. natriuretic peptides and troponin).

Beyond the primary endpoint, such a trial would be an outstanding opportunity to track the natural history of patients with genotype-positive HCM and to gather pilot information on the effects of drug on other manifestations of disease. Ultimately, the study will help provide further insight into which HCM patients are at highest risk for developing progressive adverse remodeling and arrhythmia.

II. Translational Initiatives.
There is a clear lack of fundamental insights into disease mechanisms in HCM. That is, the molecular pathways regulating the key phenotypic components of HCM discussed above are not understood, and this severely limits options for therapeutic intervention. These molecular pathways should be thoroughly explored to identify novel translational opportunities. The following strategies should be considered:

**A. Preventing mutant gene expression.**

**Recommendation: Develop strategies to reduce or eliminate expression of disease-causing alleles.**

While pharmacologic therapies may alleviate symptoms in patients with HCM, and some day could retard the progression of disease, such therapies would likely only serve a palliative function and may not meaningfully impact patient survival. A more direct approach would be to develop strategies that significantly reduce or eliminate expression of the HCM disease-causing allele. Given the state of developed technology at the current time, such an approach would have to rely on very subtle differences in nucleotide sequences between a “good” copy of an allele and the “mutant” copy, as most HCM mutations cause a missense or nonsense mutation in only one allele of sarcomere genes. These missense mutations are thought to generate a dominant acting protein product that alters the contractile parameters of myofilaments in the heart. Thus, one strategy could be to selectively silence expression of the mutant allele without affecting the expression of the wild-type allele.

While this strategy is indeed technically very challenging, the recent advent of siRNA/shRNA and miRNA approaches may offer a unique opportunity to selectively reduce expression of a mutant allele over a wild-type allele. MicroRNAs are small 19-25 nucleotide sequences that are resident in the genome and are transcribed for the sole purpose of specifically targeting subsets of mRNAs to downregulate their expression or promote their degradation. Artificial siRNAs take advantage of the same general process and intracellular machinery to selectively reduce expression of mRNAs. Thus, it is conceivable that an siRNA or similar anti-sense RNA sequence could be generated that distinguishes between a mutant and non-mutant allele at the level of mRNA. Such sequences could target the disease causing mutation itself, or other silent mutations (single nucleotide polymorphisms) that are non-disease causing but still distinguish the
mutated allele from the normal. Perhaps the most challenging hurdle would be to generate a proper siRNA that selectively targets the mutant allele, and to rigorously validate it in some sort of cell-based assay (see below for use of induced pluripotent stem (iPS) cells for this and other purposes). Other significant hurdles would include delivery of said siRNA to the hearts of patients (though this has been accomplished to some extent in animal models), and maintenance of siRNA levels chronically so that mutant gene expression is silenced long-term (and this has also been achieved to some degree with antagonists of miRNAs, so-called antagonirs.\textsuperscript{70}

Other approaches with sequence-specific oligonucleotides, such as catalytic RNAs that also target mutant mRNA, or even zinc-finger directed nucleases that could selectively target the mutant allele at the level of the DNA, should also be considered. Indeed, zinc-finger-directed transcription factor nucleases can selectively target DNA sequences in mammals as a means of achieving gene deletion or even correcting a point mutation.\textsuperscript{71} The concept would be to target polymorphisms anywhere within the DNA of the gene itself that are specific to the disease allele, as a means of selectively inactivating it, or even selectively changing the mutation back to the non-mutant sequence. Proof-of-concept for such an approach in cells and animal models, and delivery of these alternate platforms would also be considerable hurdles to overcome. Regardless of these and other technical obstacles, approaches, such as those discussed here, have emerged that might render themselves to treatment of HCM and offer a long-term therapy that would delay disease progression in a meaningful manner.

**B. Characterization of the evolution of HCM phenotypes**

**Recommendation:** Develop new research initiatives to define novel approaches to prevent or delay the development of pathological HCM phenotypes. Our view is that HCM mutations cause pathological phenotypes by activating specific signaling pathways. It is important to utilize existing and new mouse models of HCM to better characterize the evolution of the HCM phenotypes and the pathways regulating them, and to test whether these are also activated in HCM patients. These studies should identify the aberrant activation of those signaling cascades that ultimately induce pathological remodeling and altered mechanical and electrical function. We believe that the focus
should be placed on knock-in models rather than transgenics since they appear to more closely recapitulate human disease and maintain protein-protein interactions that knock-outs cannot. We propose the following research directions:

1. **Identify intracellular signaling cascades activated in HCM and determine if they induce the associated pathological phenotypes.** There are good examples of success of this strategy in the pre-clinical literature. One example is the mouse model in which HCM is induced by transgenic expression of human MYH7 mutations. These animals show many of the clinical manifestations of human HCM. Treatment of these animals with Ca\(^{2+}\) channel antagonists can prevent the HCM phenotype, suggesting that Ca\(^{2+}\) influx is abnormal in these animals and somehow this induces hypertrophy and associated dysfunction.\(^{41,42}\)

Since strategies to prevent expression of the mutant allele are at least a few years away, new studies should establish links between HCM mutations and the activation of those signaling pathways that ultimately induce HCM phenotypes. These studies should attempt to identify those HCM-activated signaling cascades that cause the electrical and mechanical disturbances that produce premature death in HCM. They should identify the signaling pathways in HCM that initiate hypertrophy, arrhythmias, cardiomyocyte apoptosis and necrosis, remodeling of the extracellular matrix, and abnormal Ca\(^{2+}\) handling. These studies could employ both targeted signaling pathway (i.e. “best guess”) approaches based on prior studies (reviewed in \(^{72,73}\)) and unbiased proteomic analyses. Translational studies should then be designed to interrupt putative HCM-inducing signaling cascades to determine if disease phenotypes can be prevented or delayed.

HCM phenotype-inducing or perpetuating signaling pathways identified in animal models should ideally be studied in samples from human HCM hearts to validate candidate pathways for novel therapies. An exploration of fundamental signaling pathways and transcript profiles (see below) in biopsy specimens from HCM patients would allow not only a determination as to whether transcriptional and pathway profiling findings in mouse HCM models are recapitulated in patients, but could also allow an identification of novel candidate nodal molecules that are disease-promoting or disease-associated factors. Subsequent manipulation of these key nodal molecules in mouse
models would then allow a determination of their potential promise as drug targets. These could also be tested in larger animal models of HCM including in the rabbit and pig.

Limiting our ability to achieve the above goals is the necessity of obtaining appropriate human LV tissue samples, including from patients who are in the earliest phases of phenotypic expression of disease. It is hoped that novel and safe approaches for percutaneous acquisition of myocardial tissue can be developed so that such samples can be obtained.

2. Develop approaches to more fully characterize the development of diastolic dysfunction in HCM model systems. These studies might first focus on identification of sarcomere mutations that may be associated with more severe diastolic dysfunction that results in the development of significant restrictive physiology. Studies to link mutations associated with prominent diastolic dysfunction to putative initiating mechanisms including fibrosis and extracellular matrix remodeling, myocyte hypertrophy, myofilament abnormalities, Ca\(^{2+}\) dysregulation, impaired mitochondrial function, oxidative stress, myocardial ischemia, and altered energetics should then be done. Indeed, given the pervasiveness of diastolic dysfunction in patients with HCM, the latter studies to identify mechanisms underlying the development of diastolic dysfunction should be done irrespective of whether strong genotype/phenotype correlations are found. Specific approaches to examine these issues are further addressed in Basic Research initiatives.

3. Develop new model systems with less common genetic variants. While it has been assumed that the pathophysiology is similar in many HCM mutations, this is likely not the case. Therefore, additional mouse models of human mutations should be created and their pathophysiology defined. As more thorough genetic evaluations become commonplace, additional mutations will be identified. Mouse models of these could lead to a new understanding of the pathophysiology of HCM.

C. The burden of arrhythmia is an important component of the pathophysiology of HCM, but factors regulating arrhythmogenesis in HCM are not clear. Recommendation: Define the pathophysiology of arrhythmias and the arrhythmia burden in patients with HCM.
1. Develop reliable methods to study sudden death, including exercise-induced sudden death, in HCM model systems so that critical initiating factors can be identified and therapeutic interventions can be tested. This research direction is driven by the lack of an in-depth understanding of mechanisms leading to sudden death in patients with HCM. Similarly, the pathogenesis of ventricular tachyarrhythmias, the fundamental cause of sudden death in HCM, is not clear. Ventricular tachyarrhythmias have been proposed to be secondary to myocardial ischemia, replacement fibrosis, abnormal calcium handling by HCM myocytes, and other factors, but knowledge of the preponderance of one or multiple factors and whether they are direct or indirect trigger events, or simply bystander effects, is uncertain.74 Better definition of the substrates for ventricular arrhythmias could improve risk stratification for primary prevention of SCD.

Despite limitations intrinsic to the discrepancies between humans and mice related to heart rate, heart size, and repolarization (mostly K) currents, mouse models are an important tool because they offer the possibility of assembling an integrated understanding of arrhythmia mechanisms- from ion channels, to cellular arrhythmia surrogates (after-depolarizations), intercellular coupling, and isolated heart and in vivo arrhythmias.

2. Study mechanisms of arrhythmogenesis in mutation-specific iPS cells and in cells isolated from mouse models.

Dissecting mechanisms of arrhythmogenesis at the molecular and cellular level in HCM also remains a major challenge. These challenges arise, in part, because of the variability in genotypes and phenotypes, and the likely requirement that final common effectors (ion channel and associated macromolecular complexes and signaling molecules) must operate in the cellular context of the hypertrophied cardiomyocyte.

Induced pluripotent stem (iPS) cells are cells that behave as stem cells but are derived from fully differentiated somatic cells, typically fibroblasts. This process involves a variety of protocols that require viral transduction or plasmid transfection of one to four transcription factors (Oct3/4, Sox2, c-Myc, Klf4 or, alternatively, Oct4, Sox2, Nanog and Lin28) with or without treatment with small molecules.75-78 These iPS cells, including those derived from human fibroblasts, can then be differentiated into several lineages, including cardiomyocytes that genotypically and phenotypically mimic adult
cardiomyocytes\textsuperscript{79, 80} Thus it is possible to create patient-specific cardiomyocytes to study in culture with minimal discomfort and risk to the patient. Thus one could theoretically study the biology (or physiology, see below) of cardiomyocytes “ex vivo” with specific HCM mutations. This approach has obvious appeal, albeit with caveats such as uncertainty as to how faithfully the iPS-derived cardiomyocytes recapitulate cardiomyocytes in situ. Of importance, the mutation-specific iPS cells represent a novel context for measuring the electrical remodeling processes at the level of action potentials, intracellular Ca\textsuperscript{2+} handling and ionic currents. Specifically we propose:

a. An examination of alterations in ion channels employing a biophysical and pharmacological dissection in iPS-derived cardiomyocytes and in adult cardiomyocytes isolated from mouse models.

b. An examination of disturbances in intracellular Ca\textsuperscript{2+} that may be central to arrhythmogenesis in HCM. It is hoped that these studies in iPS cell may allow an identification of intrinsic abnormalities of the myocytes in the absence of the complicated in vivo milieu.

3. Develop HCM model systems with atrial tachyarrhythmias so that fundamental mechanisms underlying these arrhythmias can be identified and novel therapeutic approaches can be tested. Factors driving atrial arrhythmogenesis are not well-defined, and studies to date have been largely correlative. Longitudinal studies indicate that disease duration and atrial enlargement increase the risk for AF, an arrhythmia that occurs in at least 20\% of HCM patients and contributes to thromboembolic events and sudden death.\textsuperscript{31} Diastolic dysfunction and left ventricular outflow tract obstruction contribute to atrial remodeling in HCM, but whether invasive strategies to reduce obstruction can attenuate AF is not known.

4. Assess arrhythmia burden in patients with HCM.

In addition to trying to understand the pathophysiology of arrhythmia generation with mouse models, it is apparent that the burden of arrhythmia, types of arrhythmias, and factors correlating with arrhythmias in patients with HCM is unclear. We suggest that implantable arrhythmia monitoring in patients be employed to explore the relationships between specific arrhythmias and genotype, phenotype, and biomarker expression.
Arrhythmia ascertainment is a significant challenge for HCM studies. Conventional, short term ambulatory (Holter) monitoring devices and event recorders cannot capture most arrhythmias because of their short duration of usage and the infrequent periodicity of arrhythmias in most HCM patients. Furthermore, non-implantable arrhythmia monitors are subject to electrical noise and mechanical failure (e.g. lead dislodgement). Some devices require activation by the patient during symptoms, and therefore may fail to record asymptomatic arrhythmias or prove to be technically challenging for some patients.

There is clear evidence that implantable cardiac defibrillators (ICDs) can benefit some patients with HCM. Current guidelines support the use of ICD therapy in patients with one or more of the high risk clinical characteristic discussed above. The emerging population of HCM patients with ICDs represents a unique opportunity for monitoring, phenotyping and defining the natural history of arrhythmias, (at least in this higher risk population) due to the dual function of ICDs as therapeutic tools (i.e. defibrillators and anti-tachycardia pacing) and observational instruments (i.e. data storage devices for arrhythmias). ICD detected arrhythmias have been used in clinical studies to assess antiarrhythmic efficacy of fish oil, proarrhythmic risk of dofetilide, and ICD efficacy in HCM. ICD detected and stored arrhythmias could be important for developing associations between arrhythmias and specific phenotypes, biomarkers and/or gene expression profiles. Ultimately, these associations could lead to new mechanistic understandings of the pathophysiology of arrhythmogenesis in HCM and improve risk stratification of HCM patients.

A more recent development in long-term monitoring of patients is the implantable arrhythmia monitor. These small MRI-compatible monitoring devices are implanted in the subcutaneous tissue of the chest and can monitor for arrhythmias for up to 3 years. In the broader population, these devices are primarily used in the evaluation of patients with syncope of unclear etiology, to detect AF, and to monitor AF burden. These devices could also be used in HCM patients to explore relationships between arrhythmias, genotype, phenotype, and biomarkers in patients who do not qualify for ICD placement (i.e. patients at lower risk for sudden death). They could also be used to determine the incidence and epidemiology of ventricular tachyarrhythmias and of AF. It is hoped that
the approaches outlined above will allow for the generation of more refined criteria for ICD use, a better understanding of mechanisms driving AF, and a clearer delineation of risk factors/markers for atrial and ventricular arrhythmias in HCM patients.

D. It is uncertain at this time whether emerging findings from transcript profiling in mouse models of HCM are mirrored in patients.
Recommendation: Identify transcript profiles in mouse models that regulate expression of the various HCM phenotypes and, if possible, compare those to profiles in patients.

Comprehensive strategies have been developed to profile transcriptional responses to HCM mutations. By profiling myocytes and myocardial tissues from HCM models from the pre-hypertrophic phase and throughout disease development, these approaches can define the earliest cellular events triggered by a gene mutation as well as more longitudinal responses. While model organisms provide critical data that would be almost impossible to obtain from patients, direct study of human HCM tissue is also needed to understand the impact of different causal mutations, background genotypes and lifestyle on pathophysiology.

Interrogation of comprehensive transcriptional datasets by gene ontology analyses could potentially identify biological processes and molecular functions that are activated in the HCM hearts from model organisms and patients. In concert with knowledge-based networks to identify molecular interactions, bioinformatic platforms can identify putative molecules involved in the progressive stages of HCM pathology. Molecular or pharmacologic manipulation of candidate molecules in HCM models, when correlated with pathologic phenotypes, can define critical factors that contribute to sarcomere dysfunction, myocyte growth, myocyte survival or death, or myocardial fibrosis. When combined with platforms that integrate drug interactions to molecular networks, there are outstanding opportunities for the development of mechanistic-based therapeutic strategies in HCM.

E. Use of iPS cells to study HCM mechanisms.
Reccomendation: Create patient-specific cardiomyocytes for study in culture.
iPS cells were described above. Despite caveats, we believe that validated iPS cell-derived human cardiomyocytes should be created for common HCM mutations and should be made available to researchers in order to determine the validity and potential utility of this approach. In addition to their potential value in studying arrhythmogenesis, the physiology and biology of the HCM myocytes carrying various mutations could be examined in isolation (thereby minimizing confounders such as neurohormonal inputs, signals from the matrix, etc. and without the “history” of the in vivo pathology).

III. Basic Initiatives

Introduction. Study of the fundamental mechanisms that are causal in HCM remains an essential component in the quest to diagnose and cure the disorder. Nearly 50 years after the identification of HCM in young adults as an autosomal dominant disease,\textsuperscript{88} and 20 years after its linkage to sarcomeric protein mutations\textsuperscript{89}, we still do not understand the most proximal mechanism(s) that initiates the disorder. There are hundreds of sarcomere mutations linked to HCM.\textsuperscript{90} Approaches to this vast array of mutations must involve a search for common disturbances in the multiple roles of sarcomere proteins in linking metabolic support mechanisms to the dynamics of force generation, maintenance, and relaxation, as complex responders to Ca\textsuperscript{2+} signaling, and as dominant Ca\textsuperscript{2+} buffers, as determinants of the diastolic state. Given the central role of altered Ca\textsuperscript{2+} signaling in regulating pathologic hypertrophy and ventricular remodeling in other disease models, the mechanisms described above may well drive the phenotypic expression of HCM.

Sarcomere proteins are also directly engaged in signal reception, transmission, and transduction, even in the absence of effects on Ca\textsuperscript{2+}.\textsuperscript{91,92} Sarcomere signaling involves mechanical feedback through the costamere, integrins, and the extracellular matrix, as well as to the nuclear envelope. Titin and the Z-disk network are also sites of docking of kinases (e.g. PKC), phosphatases (eg. calcineurin), and transcription factors (e.g. MLP), which shuttle back and forth to their sites of regulation in complex pathways. There is little doubt that further investigations of these signaling networks will reveal new and significant aspects of their role in excitation, contraction and relaxation, and in adaptive and maladaptive responses of heart muscle.
We also do not understand how the sequelae of these initiating mechanisms promote the progression to hypertrophy and sudden death. More studies are required, which include measurements at time points in the progression of the disorders with emphasis on the role of altered perfusion, altered metabolic support mechanisms, abnormalities in the ECM, and abnormalities in electrical excitation and conduction, all of which are addressed below. The involvement of post-translational modifications (PTM) and which of these PTMs ameliorate, and which exacerbate, progression of the disorder remains poorly understood. The role of sarcomere mutations and altered shuttling of kinases, phosphatases, and transcription factors in the progression of the disorder also remains poorly understood. It is apparent that rather than serial studies of the long list of individual mutations, now is the time for more in depth investigation at the basic level of a short list of prevalent and penetrant models expressing a sarcomere protein linked to HCM.

A. Recommendation: Delineate initiating stimuli that are recruited in response to expression of mutant sarcomere proteins.

1. Are alterations in Ca\(^{2+}\)-fluxes the initiating stimulus for pathological hypertrophy and disease? How mutations in sarcomere proteins induce pathological hypertrophy is still not clearly defined. A straightforward hypothesis is that these mutations induce abnormalities in myocyte Ca\(^{2+}\) handling that activate pathological hypertrophy signaling cascades. This idea is strongly supported by studies in HCM mice showing that treatment with Ca\(^{2+}\) channel antagonists partially blocks the development of pathological hypertrophy.\(^{41,42}\) There is also an increased sensitivity of sarcomeres to Ca\(^{2+}\) in many sarcomere mutations\(^{90}\) in which more cross-bridges are engaged in force and shortening than in the normal heart, and energy consumption is diverted to support the sarcomeres and away from support of Ca\(^{2+}\) uptake.\(^{93}\) These mutation-induced changes in Ca\(^{2+}\) signaling could be a primary stimulus for multiple pathological cellular processes. These mechanisms need to be explored in appropriate model systems in which in-depth analysis of cellular Ca\(^{2+}\) fluxes can be carried out. Given the fact that cytosolic free [Ca\(^{2+}\)] is influenced by Ca\(^{2+}\) binding to myofibrils, experiments exploring abnormalities
in Ca\textsuperscript{2+} handling in HCM need to be performed in conditions in which the force bearing state of the myofibrils can be determined.

There is also evidence that the HCM phenotype can be rescued by manipulation of the Ca\textsuperscript{2+} handling properties of HCM myocytes (i.e. viral aided gene transfer of SERCA2a in neonatal hearts expressing mutant tropomyosin).\textsuperscript{94} This idea is deserving of additional study to see if results can be translated into animal models and then, hopefully into humans. In these studies, Ca\textsuperscript{2+} handling should be manipulated at multiple levels, from influx pathways to sarcoplasmic reticulum (SR) storage and release, to identify the most reliable strategy to inhibit the development of pathological phenotypes without negatively impacting cardiac function. New studies are also needed to define how altered Ca\textsuperscript{2+} fluxes on and off of troponin C (TnC) change the kinetics of the cytosolic free [Ca\textsuperscript{2+}] transient and what role this may play in arrhythmogenesis. In HCM, increased Ca\textsuperscript{2+} binding by TnC has been correlated with a propensity toward arrhythmias\textsuperscript{95} and this could be related to abnormalities in Ca\textsuperscript{2+} handling.

The complexity of the potential role of altered Ca\textsuperscript{2+} fluxes is exemplified by studies of patients and animal models harboring mutations in TnT. Patients and mouse models demonstrate little or no hypertrophy. Yet there are apparent alterations in Ca\textsuperscript{2+} fluxes in myocytes isolated from these models.\textsuperscript{96}

Collectively these studies suggest that mutations in myofibrillar proteins often involve reactive changes in Ca\textsuperscript{2+} signaling that are likely to be required to ensure the pump function of the heart. If and how these changes in Ca\textsuperscript{2+} handling contribute to the associated hypertrophy and eventually cardiac dysfunction is an area that needs much more study.

2. Do alterations in perceived stress/strain relations at the level of the sarcomere initiate hypertrophy and disease? With the many studies identifying an increase in Ca\textsuperscript{2+} sensitivity with HCM-linked mutations in the sarcomeres, came the reasonable hypothesis that with the increased stress, there is impaired relaxation leading to increased strain and diastolic dysfunction. Diastolic stretch is well known to lead to hypertrophic signaling, growth of myocytes and maladaptive remodeling including electrical abnormalities.\textsuperscript{97, 98} Altered stress would be expected to strain titin and the Z-disk network, which may alter docking sites and trafficking of proteins involved in critical
homeostatic processes such as protein phosphorylation and signaling to the nucleus. This potential initiating event has not been a primary focus of studies in most models of HCM. The linkage of mutations in Z-disk proteins to HCM provides a clue that this may be an important primary causal mechanism. Evidence that HCM-related mutations enhance sarcomere response to Ca\(^{2+}\) led naturally to the question of whether a reduction in Ca\(^{2+}\)-sensitivity would offset the sequelae in animals. Preliminary studies employing cross-breeding of mouse models of HCM linked to a tropomyosin mutation, with a model expressing constitutively desensitized myofilaments has demonstrated robust rescue of the phenotype. These data strongly indicate the need to search for small molecules or other approaches that reduce myofilament Ca\(^{2+}\) responsiveness and to test for their effectiveness after the onset of HCM.

Post-translational modifications of sarcomere proteins may also alter the initiating mechanism as is the case with PKC-dependent phosphorylation of TnI in an HCM model linked to a mutation in TnI. The potential effects of other PTMs such as oxidation, methylation, nitrosylation, and acetylation have not been generally explored. PTMs associated with increased sympatho-adrenergic activity seem especially important to understand. Sudden death in young athletes needs to be better understood in terms of the increased sympatho-adrenergic activity and increased serum catecholamines. This mechanism itself induces hypertrophy, and the relative role of underlying HCM needs to be better understood. There is a greater prevalence in sudden death in young male athletes, and the incidence in pre-pubertal athletes appears less frequent. Thus, issues related to the effects of androgen- and estrogen-related signaling networks needs further study in mouse models.

**B. Recommendation: Investigate alterations in metabolism in HCM.**

1. Characterize any alterations in metabolism and determine whether correcting these can prevent or rescue the hypertrophic phenotype. Studies in the past decade have consistently demonstrated that myocardial energy metabolism is impaired in HCM. In patients harboring mutations of β-myosin heavy chain, cardiac troponin T, or myosin-binding protein C, direct measurement of cardiac energetics by \(^{31}\)P NMR spectroscopy showed a ~30% reduction of phosphocreatine to ATP ratio (PCr/ATP) suggesting a
significant depletion of energy reserve in the heart.\textsuperscript{104, 107} Importantly, this impairment occurred independent of the specific mutation or the presence of cardiac hypertrophy.\textsuperscript{104} Similar observations have been made in transgenic hearts expressing a variety of mutant proteins.\textsuperscript{106, 108} Furthermore, by performing \textsuperscript{31}P NMR spectroscopy of isolated perfused mouse heart, it is possible to measure systolic and diastolic function simultaneously with assessment of cellular energetics and thermodynamic status. In these studies, impaired energetics was found to precede cardiac hypertrophy and systolic dysfunction but coincided with the development of diastolic dysfunction.\textsuperscript{108} The depletion of energy reserve compromises the thermodynamic efficiency of ATP hydrolysis (i.e. reduces the free energy release of ATP hydrolysis).\textsuperscript{109} Such a reduction impairs the function of some high free-energy requiring ATPases, such as SERCA2a, myosin ATPase and Na\textsuperscript{+}/K\textsuperscript{+} ATPase, all critical to myofibril contraction-relaxation and ion homeostasis.\textsuperscript{109-111} This raises the speculation that defects in energetics may contribute to the diastolic dysfunction and arrhythmias often observed in this disease.

Either increased energy expenditure or reduced energy supply can account for the lower energy reserve in the heart. Altered myofilament function caused by the mutations (e.g. altered calcium sensitivity of the myofilament, impaired actin-myosin interaction, and increased myosin ATPase activity) has been shown in a number of recent studies.\textsuperscript{112-114} These defects increase energy demand or decrease energy efficiency in myofibril contraction, and thus may constitute an important mechanism for impaired myocardial energetics. On the energy supply side, mitochondrial dysfunction and altered substrate metabolism have also been reported in animal models and patients.\textsuperscript{107, 115, 116} However, these changes are not observed in every mutant model.\textsuperscript{117} It is also important to distinguish the primary versus the secondary changes in cardiac metabolism due to severe cardiac fibrosis and heart failure in this disease. As highlighted in several sections of this document, studies in the early stage of the disease before the development of a full-blown cardiomyopathy are of critical importance.

Although the impairment of energy metabolism has been widely observed in HCM, its role in the pathogenesis and progression of the disease has not been defined. Very limited information about cardiac energetics in patients with familial HCM is available and none of the data were collected from patients at early stages of the disease.
It is not clear whether the decreased energy reserve in the heart of hypertrophic cardiomyopathy patients is a primary abnormality or secondary to myocardial remodeling. Thus, it will be extremely informative to include a $^{31}$P NMR spectroscopic (MRS) exam in clinical studies of these patients especially in those studies designed to target the early stage of the disease (e.g. Clinical Track, Sections B and C). Currently, MRI has been used clinically to monitor cardiac hypertrophy and ventricular function. A combined MRI-MRS approach will be even more powerful as it integrates the physiology with biochemistry in a non-invasive setting. This will not only provide a longitudinal observation of changes in myocardial energetics in the natural course of the disease it will also determine the relationship between the responses in myocardial energetics and the efficacy of a therapy.

There is now accumulating evidence suggesting that impaired energy metabolism is a common feature in most of the cardiomyopathies caused by mutations of sarcomere proteins. This may represent an excellent target for therapeutic interventions once we determine the energetic element is critical for the progression of the disease. In animal models where changes of myocardial energetics have been observed before the development of hypertrophy and failure, there is a good opportunity to determine whether interventions that either improve energy efficiency or increase energy supply rescue the cardiomyopathy phenotype. One central question will be whether a normalized cellular energetic status improves ion pump function and reduces the risk of arrhythmia. Moreover, strategies that reduce the adverse consequences of depleted energy reserve by reducing the energy demand and stabilizing E-C coupling, such as calcium channel blockers, warrant further investigation.

2. **Determine if altered sarcomere responses to Ca$^{2+}$ are linked to altered metabolism.** Altered metabolic support for cellular process has been demonstrated in HCM, and there are mutations in prominent metabolic control enzymes such as AMP kinase that are linked to HCM phenocopies. Recent evidence demonstrates linkages among control elements in metabolism and sarcomere function. For example, protein kinase D and AMP kinase not only regulate metabolism, but also modify sarcomere response to Ca$^{2+}$ by direct phosphorylation of sarcomere proteins, most likely, TnI.
Thus the possibility exists that modifications in a kinase that regulates metabolism may also alter sarcomere response to Ca\(^{2+}\) much as is the case with sarcomere mutations. It is also significant that the switch in HCM from lipids as a preferred substrate to glucose may result in altered lipid signaling, which may also affect sarcomere response to Ca\(^{2+}\).\(^{120}\) The challenge to our understanding of adaptive and maladaptive control mechanisms is to fully integrate these signaling processes at the level of sarcomere proteins with elements controlling redox state, glycolysis, and oxidative phosphorylation.

3. Combine HCM models with models modifying Ca\(^{2+}\) handling, sarcomere response to Ca\(^{2+}\), and metabolism. The purpose of the studies proposed herein is to determine whether any of the above models can partially rescue the phenotypic expression of disease. The underlying hypothesis that variations in Ca\(^{2+}\) release rates or buffering capacity from mutant sarcomere proteins initiates HCM could in theory be validated if appropriate mouse models were employed to secondarily alter Ca\(^{2+}\) in a compensatory manner. For example, the MyHC 403 mutant mouse model was shown to have prolonged Ca\(^{2+}\) adherence to the contractile machinery,\(^{41}\) which was thought to induce disease through altered diastolic Ca\(^{2+}\) sensing/buffering. Thus, crossing the 403 mutant model with another mouse model that has the opposite effect of hastening Ca\(^{2+}\) release from the myofibers might rescue disease and firmly establish the Ca\(^{2+}\) hypothesis. Indeed, crossing the MyHC 403 mutant model with a TnI mutant model that also shows the same sort of Ca\(^{2+}\) disturbance led to synergistic disease and neonatal lethality in mice.\(^{122}\)

C. Recommendation: Define the role of the vasculature in HCM pathophysiology

Capillary density in the heart is tightly coupled to cardiomyocyte growth during development.\(^{123-126}\) This program is likely recapitulated in the adult heart subjected to injury or physiologic stimulation to ensure adequate perfusion as the myocyte compartment expands, and inhibition of this “matching” effect in microvessels during hypertrophy was recently shown to promote decompensation.\(^{127-129}\) More specifically, micro-vessel expansion has been reported during pressure overload myocardial hypertrophy in multiple species, including humans.\(^{124, 128, 130, 131}\) However, long-standing
hypertrophy, especially in adult humans, has been associated with a decrease in capillary density, cardiac decompensation, myocyte death and replacement fibrosis.\textsuperscript{124, 132} Abnormalities of the vasculature in HCM are associated with evidence of ischemia, and this is presumed to lead to disease progression. Future studies to examine what role vascular abnormalities play in the phenotypic expression of disease are clearly warranted.

Angiogenesis in the adult heart is likely regulated by two distinct mechanisms (with some potential overlap). The first mechanism is the well-known hypoxia/ischemia-induced response through HIF-1-\(\alpha\),\textsuperscript{133-135} and the second mechanism is associated with a general “stress-response” of the heart following acute hemodynamic overload or exercise/pregnancy-induced hypertrophy.\textsuperscript{124, 128, 130, 131, 136, 137} Such a response permits the myocardium to coordinate an increase in microvessels with the ensuing hypertrophic response, despite the absence of overt tissue ischemia. Loss of either mechanism would lead to dysynchrony in vessel and capillary content based on the perfusion requirements of the heart, thereby potentially exacerbating HCM. Future studies that investigate the molecular mechanisms underlying angiogenesis in the HCM heart are warranted, especially since ischemia, driven by both pathologic remodeling of the vasculature and impaired angiogenesis, may well be a key to phenotypes.

D. Recommendation: Identify the contribution of non-myocytes and the matrix in promoting HCM phenotype expression.

As described above, HCM presents with a complex pathology including myocyte hypertrophy and disarray, interstitial and replacement fibrosis, and dysplastic intramyocardial arteries and capillaries. Recent studies have suggested that the clinical manifestations of HCM and other forms of pathologic hypertrophy do not always correlate with myocyte-related pathologies. Rather, some phenotypic expressions of HCM may correlate more with altered functions of non-myocytes including fibroblasts and myofibroblasts. The molecular link between sarcomere mutations, fibrosis, diastolic dysfunction, and arrhythmias is not yet understood but may be critical to progression of disease.

The interstitial fibrosis associated with HCM is most prominent around arteries and in the subendocardium, suggesting that both perivascular and subendocardial fibrosis
may originate from the endothelial compartment. Recent studies demonstrate that coronary endothelial cells and endocardial cells can undergo endothelial-to-mesenchymal transition (EndMT),\textsuperscript{48, 138, 139} causing smooth muscle hyperplasia and perivascular fibrosis in mouse models of cardiac fibrosis. Furthermore these processes appear to be regulated by BMP7 (inhibiting EndMT and fibrosis) and TGFβ1 (promoting EndMT). Whether EndMT links vascular dysplasia with fibrosis in HCM is not yet known but needs to be explored. If so, modulating BMP7/ TGFβ1 signaling could provide a novel strategy to inhibit fibrosis.

In summary, despite its prominent occurrence, extra-cardiomyocyte manifestations of HCM have been largely neglected. A full understanding of the mechanisms that mediate fibrosis and vascular remodeling in HCM could prove critical to our understanding of the pathogenesis of the disease.
Supplemental References


