In recent years, the main focus of human genetic studies on hypertrophic cardiomyopathy (HCM) switched from discovering novel genes and defining disease-causing mutations to studies of mutation distribution in disease populations. Eventually these studies will define genotype–phenotype relationships, which may provide clues to understanding the disease process and help to select the most appropriate treatment strategy. Animal models engineered to recapitulate human disease provide a unique tool to investigate the pathogenic mechanisms and evaluate potential therapies. In this review, we present the spectrum of clinical HCM in the context of the genetic heterogeneity of this common human disease. Recent progress made in understanding molecular pathways that result in cardiac hypertrophy and the factors that modify these processes are discussed.

MOLECULAR GENETICS OF HUMAN HYPERTROPHIC CARDIOMYOPATHY

Hypertrophic cardiomyopathy (HCM), once considered ‘idiopathic’, is now recognized to result from dominant mutations in genes encoding the proteins of the contractile apparatus. Mutations in genes encoding cardiac myosin heavy chain (βMHC), cardiac myosin binding protein C (MyBPC), cardiac troponin T (TnT), cardiac troponin I (TnI), α-tropomyosin (αTM), essential and regulatory light chains, and cardiac actin (1–3); are well-established causes of the disease (Fig. 1). Morphologic features of HCM include ventricular hypertrophy, which is usually asymmetric and, particularly involves the interventricular septum, and atrial enlargement (Fig. 2A). Microscopic examination shows myocyte hypertrophy, myocyte disarray and often increased amounts of interstitial fibrosis (Fig. 2B and C) throughout the myocardium.

Prospective screenings of HCM populations often report mutation detection in sarcomere proteins in only ~60% of study subjects (4, 5; http://www.cardiogenomics.org). This low detection rate may in part reflect limitations of indirect screening methods, such as denaturing high-performance liquid chromatography (DHPLC) (6) or single-strand conformation polymorphism (SSCP) (7), incomplete DNA analyses of regulatory and intron sequences, uncommon genetic mechanisms [i.e. mosaicism (8)], as well as defects in unknown disease genes (which may be related to sarcomere function). Recent reports have suggested that disruption of additional proteins related to sarcomere assembly and function, including titin (9), α-MAC (1) myosin light-chain kinase (10) and muscle LIM protein (11), may also lead to an HCM phenotype. But genetic studies of cardiac hypertrophy, presumed to be HCM, have also uncovered clear evidence of clinical misclassification.

Besides well-known causes of secondary hypertrophy, such as results from hypertension and aortic valve disease, a phenotype mimicking HCM may also occur from defects in mitochondrial metabolism (12) and from storage diseases. Fabry’s disease, an X-linked lysosomal disorder due to α-galactosidase mutations (13), can be manifested as a predominantly cardiac phenotype, affecting adult men. Cardiac hypertrophy associated with pre-excitation and progressive conduction system delays has also recently been shown to result from mutations in PRKAG2 (14–16), a γ2 subunit of AMP kinase that plays an important role in controlling cell energy status as well as in regulating glycogen metabolism. PRKAG2 mutations upregulate AMPK activity and give rise to a new type of glycogen storage disease (16), manifested by cardiac hypertrophy mimicking HCM, but in association with ventricular pre-excitation and progressive conduction system disease. Although cardiac hypertrophy is a shared feature of these diverse human disorders, it is important to distinguish these states because of differences in prognosis and therapy. Throughout this review, HCM indicates sarcomere protein gene mutations that cause left ventricular hypertrophy, histopathology of myocyte enlargement and disarray, with associated replacement fibrosis (Fig. 2C) in the absence of infiltrative or storage processes.
THE CLINICAL COURSE OF HCM AND ITS VARIABILITY

The cardiac phenotype of HCM shows great diversity in the degree and pattern (asymmetric, concentric, apical) of hypertrophy (17), penetrance (18,19), age of onset and clinical course (20–22), in particular regarding heart failure and predisposition to sudden death. With age, the hearts of affected individuals undergo further remodeling manifested by changes in the chamber size and the extent of hypertrophy. A small subset of affected individuals progress to left ventricular dilatation associated with end-stage heart failure (sometimes termed ‘burned-out’ HCM) (1,23).

Variability in the clinical course is explained in part by the different roles that mutant proteins play in the sarcomere (Fig. 1) and the effect that a given mutation has on protein structure and function. The βMHC gene was the first to be identified as a cause of familial hypertrophic cardiomyopathy, and almost 100 disease-causing mutations have been defined to date (1; http://www.cardiogenomics.org). Most βMHC mutations are located in the head and head–rod junction, and some of these mutations are recognized to cause severe hypertrophy that presents clinically early in life. Although these features have led to the conclusion that all βMHC mutations cause severe disease of early onset and are associated with increased risk for outflow obstruction and sudden death, there are clear clinical distinctions between βMHC mutations Arg403Gln or Arg719Trp, which predispose to sudden death and heart failure, and missense mutations Phe513Cys, Leu908Val or Gly256Glu, which cause less severe clinical disease (1,2).

MyBPC mutations are a leading cause of late-onset HCM and are generally associated with a good prognosis (18,19). Genetic analyses of a cohort of individuals with elderly-onset HCM (24) identified no βMHC mutations but multiple mutations in MyBPC and troponin I and one missense mutation in αMHC. Hence sarcomere mutations can produce both early and severe cardiac hypertrophy, as well as late-onset, often benign disease. Although this variation in clinical consequences most likely reflects differences in the biophysical properties of mutant and wild-type peptide, factors other than these structural changes can also influence disease expression.

Evidence that other factors can modify the effects of sarcomere protein missense mutations to alter the morphologic and pathophysiologic features of clinical HCM first became evident from the study of individuals with the same disease-causing mutation (25). These analyses demonstrated that the identical sarcomere mutation in different populations can cause distinct hypertrophic morphologies and divergent clinical courses. Furthermore, significant phenotypic differences have also been observed between affected members of the same

Figure 1. HCM is caused by sarcomere protein gene mutations. Constituents of both the thin and thick filaments are mutated in HCM. Not shown are titin or α myosin heavy chain; both are rare causes of HCM. Modified with permission from Spirito, P., Seidman, C.E., McKenna, W.J. and Maron, B.J. (1997) The management of hypertrophic cardiomyopathy. N. Engl. J. Med., 336, 775–785 (23). Copyright 1997, Massachusetts Medical Society. All rights reserved.
pedigree who share one mutation. For example, a TnI Lys183Del was initially reported (26) in a proband with apical HCM, a form of focal hypertrophy that disproportionately affects the apex more than the base of the heart. The proband’s affected son had classical hypertrophic cardiomyopathy. TnI Lys183Del was also identified in 25 individuals from seven other families (27), but apical hypertrophy occurred in only one affected individual. Most surprisingly, 44% (7/16) of these 25 probands developed progressive systolic left-ventricular dysfunction, an uncommon sequela of sarcomere protein gene mutation. These data indicate that, although an HCM-causing mutation may be identified, clinical diversity can also be affected by genetic background, environment, gender and acquired conditions. These data also raise the possibility that some (genetic or environmental) modifiers account for unfavorable cardiac remodeling and predisposition to heart failure.

Human association studies have evaluated candidate molecules as genetic modifiers of HCM, particularly those that influence the extent of hypertrophy. Analyses of polymorphisms within genes encoding components of the renin–angiotensin axis, aldosterone, endothelin and tumor necrosis factor (TNF) have shown conflicting results (28–34), and appear to explain only a minor portion, if any, of the observed clinical diversity in the range of hypertrophy evident in HCM. Polymorphisms (D/I) within the ACE gene have also been reported to be associated with increased risk for sudden death (35). The complexities of defining genetic modifiers in human HCM populations remain considerable given the substantial heterogeneity of genetic causes that may independently influence hypertrophy.

The recent increase in analyses of HCM populations has revealed one unexpected genetic cause of clinical diversity. Although HCM is an autosomal dominant disorder, genetic screens have revealed a surprising number of individuals who are compound heterozygotes, i.e. when harbor two mutations in the same or different (5) sarcomere protein genes, such as βMHC and MyBPC. In addition, individuals with both a sarcomere protein mutation and a mitochondrial mutation have been reported (37) to exhibit more severe cardiac disease. These data indicate the possibility that genetic variation in sarcomere protein and other genes may exacerbate the disease course of pathologic mutations that might otherwise cause relatively benign disease.

The coexistence of other additional causes of cardiac hypertrophy (i.e. hypertension or ischemic heart disease) is presumably another cause for the variability in the ventricular hypertrophy observed in HCM. Although only a few monogenic causes of hypertension have so far been identified, recent linkage studies have identified an association between left ventricular systolic function in hypertensive individuals and

Figure 2. Anatomical features of human HCM. (A) Marked left ventricular (LV) hypertrophy is evident, with prominent involvement of the inter-ventricular septum (bar = 1.9 mm; normal < 1.3 mm). Right ventricular (RV) wall hypertrophy and left atrial (LA) enlargement is also evident. Histopathologic section stained with hematoxylin and eosin (B) reveals myocyte enlargement (hypertrophy) and cellular disorganization (disarray). Masson trichrome stain (C) shows robust amounts of interstitial fibrosis (blue) between hypertrophied myocytes (pink).
polymorphic markers on chromosome 11 (38), a region inclusive of the cardiac MyBPC gene.

Gender has been hypothesized to affect many cardiovascular disorders, including HCM. Affected women are reported (39) to have smaller ventricles, later onset of disease and a higher incidence of stroke, presumably due to atrial fibrillation (22), an arrhythmia that increases with disease duration. However, there is no significant difference in left ventricular wall thickness of males and females with sarcomere protein gene mutations. Further, the prevalence of outflow tract obstruction (which can predispose to atrial arrhythmias in HCM) or heart failure is not known to be substantially influenced by gender (40). While sudden death in HCM occurs equally in men and women, sudden death on the athletic field is almost exclusively a male event (41). Given that HCM is the most common diagnosis of these athletic field deaths (23), further study of the role of gender in these disease sequela appear warranted.

Progression of HCM to the burnt-out phase of disease represents unfavorable remodeling characterized by loss of function, wall thinning and chamber dilation that can cause heart failure, with combined systolic and diastolic dysfunction. Although this progression occurs in ~10% of individuals with HCM, there is evidence that certain populations are at greater risk for this clinical deterioration (1,42); βMHC Arg719Trp and zTM Glu180Val have been reported to particularly predispose to progressive cardiac deterioration. In addition, the burnt-out phase of HCM may be evoked by a ‘second hit’ in another gene, as has been reported in individuals with both sarcomere protein gene and mitochondrial mutations.

ANIMAL MODELS: INSIGHTS INTO MECHANISMS AND THERAPIES

Elucidation of the pathway by which distinct sarcomere protein mutations lead to the hypertrophic heart has proven difficult in humans. The development of genetically engineered animal models is increasingly providing reagents that can overcome the limitations of human research and create opportunities to investigate both disease mechanisms and potential therapies. In addition, these models can help in identifying genetic and environmental factors that modify the disease process.

One approach to creating murine models of HCM has been to introduce specific mutations using homologous recombination in embryonic stem cells. The zmHC+/-/+ mouse (43) expresses a glutamine codon instead of the normal arginine codon at position 403 in the murine cardiac myosin heavy-chain gene. The Arg403Gln mutation that occurs in the human βMHC gene causes a severe form of HCM in which ~50% of the affected individuals die before they reach 45 years old (1). zmHC+/-/+ mice have a normal lifespan when sedentary, but when subjected to extremely vigorous exercise, they develop severe hypertrophy (Fig. 3A) and some die suddenly. Heterozygous zmHC+/-/+ mice that have been inbred into SvEv strains exhibit uniform hypertrophy at 30 weeks; their left ventricular wall thickness is 1.13 ± 0.08 mm versus 0.87 ± 0.05 mm in wild type. However, when the zmHC+/-/+ mutation is crossed into the Black Swiss outbred strain, a bimodal distribution of left ventricular wall thickness is observed; only 50% of the mice develop hypertrophy. These experiments provided the first experimental evidence for a modifier gene (44) that modulates phenotypic expression of HCM.

Transgenic mice that express a compound mutation [Arg403Gln and a deletion] in zmHC (45) have yielded early insights into the effect of gender on cardiac remodeling. Aged male mice harboring the mutant zmHC transgene progress from hypertrophy to dilation (46). In contrast, female mutant mice display only cardiac hypertrophy and preserved left ventricular function.

Murine HCM models have also enabled biophysical studies of the consequences of sarcomere mutations on contractile properties. Comparable studies on human tissues were hindered not only by the variable genotypes that cause HCM (Fig. 1), but also by difficulties inherent in obtaining and analyzing human myocardium. Analyses of cardiac muscles from murine HCM models expressing mutations in zmHC, cardiac MyBPC, cardiac TnI and cardiac TnT provide evidence that these hypertrophic mutations produce enhanced contractile function at the cost of increased work and altered Ca2+ homeostasis (47–50). NMR studies of the zmHC+/-/+ mice (51) found reduced basal energy stores and unfavorable ATP/Pi ratio, which may further decrease the potential gain in free energy from ATP hydrolysis. Longitudinal in vivo assessments of cardiac physiology in mutant mice indicate that HCM defects adversely affect cardiac function and cardiovascular hemodynamics in advance of the hypertrophic phenotype (52). Even before the onset of morphologic or histopathologic findings of HCM (Fig. 3B and C), zmHC+/-/+ mice exhibit diastolic dysfunction. Once hypertrophy is evident, zmHC+/-/+ mice have enhanced systolic pressure development and increased systolic stiffness; changes occur at the expense of stroke volume (which is reduced) and work efficiency.

Cellular changes are also evident in mutant myocytes; although cytosolic calcium levels are comparable to those in wild-type hearts, the maximum calcium release capacity from the sarcoplasmic reticulum (SR) stores is significantly decreased (53). In addition, protein levels of the SR Ca2+ binding proteins calsequestrin and junctin and ryanidine receptor levels are decreased in zmHC+/-/+ mice (54). These changes, as well as increased phosphorylation of ryanidine receptor channels, are prominent in advance of the development of gross or histologic features of HCM.

The significance of altered SR Ca2+ handling in zmHC+/-/+ mutant hearts has been elucidated by comparing the hypertrophic response to pharmacologic agents that further modulate myocyte Ca2+. Administration of calcineurin inhibitors (cyclosporin A and FK506) produces increases in diastolic Ca2+ levels in wild-type but not zmHC+/-/+ hearts (53). Although the Ca2+-calcineurin signaling cascade inhibited by these agents has been demonstrated to stimulate cardiac remodeling by effecting transcriptional activation of hypertrophic genes (55), calcineurin inhibition in zmHC+/-/+ mice had a very different effect. Administration of cyclosporin A to zmHC+/-/+ mice caused marked acceleration of HCM pathology, with rapid evolution of severe, often lethal, ventricular hypertrophy (53). Co-administration of the L-type calcium channel inhibitor diazoxide prevented this exaggerated cyclosporin A response, by normalizing Ca2+ handling by mutant myocytes. Taken together, these data indicate that
dysregulation of Ca\(^{2+}\) is an important signal in producing the hypertrophic phenotype of \(\text{zMHC}^{+/403}\) hearts, perhaps reflecting increased calcium sensitivity and decreased efficiency of the mutant sarcomere. This hypothesis has been further explored by independent inhibition of the calcium L-type channel by diltiazem administered to pre-hypertrophic \(\text{zMHC}^{+/403}\) mice (54). Not only did diltiazem restore normal levels of SR Ca\(^{2+}\)-binding proteins and ryanidine receptors, but in addition diltiazem decreased the long-term development of histopathologic findings of HCM. Myocyte disarray, fibrosis and cardiac hypertrophy were all attenuated in comparison with untreated \(\text{zMHC}^{+/403}\) mice. In addition to potential therapeutic approaches indicated by these studies, these data also imply that factors that modulate myocyte Ca\(^{2+}\) levels may also have a substantial influence on phenotype expression of HCM.

Hypertrophic remodeling is a complex process that typically involves the growth and death of myocytes. As a consequence of myocyte death, interstitial fibrosis can be a prominent feature of cardiac hypertrophy, and is usually prevalent in HCM (Figs 2C and 3C). The mechanisms that account for premature death of hypertrophied myocytes are largely unknown, although signaling pathways triggered by altered calcium homeostasis (55), by increased wall stress or by increased energy demand from the hypertrophied myocardium (51) may all contribute to this process. Furthermore, because increases in cardiac fibrosis can compound poor heart function, and in particular increase diastolic dysfunction, inhibition of this process may improve function in hypertrophied hearts. Two studies have attempted to decrease collagen deposition and interstitial fibrosis in HCM models. Simvostatin caused regression of established hypertrophy in a transgenic rabbit model of \(\text{MHC}^{403}\) mutation (56), an effect attributable to interference with stress signaling and the renin–angiotensin–aldosterone system. Likewise, the angiotensin receptor blocker losartan reduced myocardial fibrosis, collagen and transforming growth factor \(\beta1\) (TGF-\(\beta1\)) in a transgenic mouse model of \(\text{TnT}^{\text{Arg92Glu}}\) mutation (57).

Altered adrenergic signaling is a common feature of many cardiovascular pathologies, including HCM (58). Indeed, the most commonly prescribed drugs for symptom relief in HCM are \(\beta\)-adrenergic receptor inhibitors. However, whether the beneficial effects of these agents (59) are directly related to their effects on the hypertrophied myocardium or due to improved hemodynamics (i.e. slower heart rate and lower systolic pressure) has been unclear from human studies. At the level of the myocyte, \(\beta\)-adrenergic stimulation might mediate excessive phosphorylation of ryanidine receptors (60) [through protein kinase A (PKA)] and thereby contribute to the maladaptive responses. Recent studies of these processes have been probed by crossing a genetic model with a dysfunctional \(\beta\)-adrenergic receptor kinase and a transgenic model of HCM. The compound mutant mouse showed an attenuated HCM phenotype and a diminished propensity to progress to heart failure (58). Although these data do not yet delineate whether adrenergic signaling affects primarily myocyte-

Figure 3. \(\text{zMHC}^{+/403}\) mice model human HCM. (A) Left ventricular (LV) hypertrophy (free wall = 1.4 mm; normal < 0.9 mm) and left atrial (LA) enlargement are evident when mice are subjected to vigorous exercise. Histopathology demonstrates myocyte hypertrophy and disarray, and increased amounts of interstitial fibrosis are comparable to what is observed in human HCM (Fig. 2). (B) Hematoxylin and eosin stain. (C) Masson trichrome stain.
dependent or independent processes, these data provide the first explanation for the palliative benefit (59) observed in patients receiving β-adrenergic inhibitors.

LIMITATIONS OF ANIMAL MODELS

Despite the exquisite capacity of murine models to recapitulate many aspects of HCM, clear differences exist between the models and human disease. First, the use of mouse models to probe the effects of mutant myosin is complicated by the predominance of different MHC isoforms in the ventricles of mice (α) versus humans (β). Second, experimental models produced by transgenic approaches may result in higher levels of mutant transcripts than levels that occur from a single mutant allele causing a dominant human disorder. In addition, regulated expression of transgenes may be quite different from physiologic expression of endogenous genes. Third, important intrinsic differences in cardiac function between species are recognized, most notably heart rate, which is 10-fold lower in humans than in mice. Finally, the effects of exercise physiology on HCM mutations may be underestimated in laboratory models. There is substantial evidence for more severe disease and adverse cardiac events (particularly malignant tachyarrhythmias leading to sudden death) in HCM patients who participate in vigorous athletics. An equivalent natural history of the disorder may be incompletely explored in the sedentary environments of most laboratory models.

The causes of other disparities, such as concentric hypertrophy in mice but asymmetric hypertrophy in humans and the propensity for 10% of human but not murine HCM to progress to develop heart failure, remain unexplained. These important differences, however, indicate a need for caution in directly extrapolating data obtained from models of HCM directly to the human disease.

CLINICAL PERSPECTIVE

The discovery of mutations in sarcomere protein genes (Fig. 1) has defined the molecular basis for HCM. Further, these data have provided a framework for understanding the signaling processes that cause cardiac hypertrophy. Whether distinct gene mutations that produce a similar cardiac phenotype trigger the same or other pathways remains an open question, the answer to which may have important therapeutic ramifications.

An expectation from successful human genetic research is often that gene-based therapy will become evident. For HCM, the complexities in achieving this goal are many. Technical complexities are considerable for establishing cost-effective, efficient gene-based diagnosis in a disorder with 10 disease alleles. (Our progress in this endeavor can be monitored at http://www.cardiogenomics.org) Further, the substantial clinical variation, from absent or mild symptoms to severe manifestations in sudden death, indicate a clear need to establish whether and to what extent genotype influences phenotype. Both of these facets require ongoing study of human HCM. In concert with continued discovery of novel approaches to abrogate disease in experimental models, the possibility of appropriate disease prevention trials in HCM is fast becoming a reality.

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